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We express our gratitude to the Maharaja Sayajirao University of Baroda for a very generous donation given towards the cost of the printing and publication of this Journal for the year 1960-61.

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ANNOUNCEMENTS

The Second International Congress of Radiation Research will be held at Harrogate, Yorkshire, England, August 5th–11th, 1962. It is sponsored by a committee set-up at the First Congress at Burlington, Vermont, in 1958, and by the Association for Radiation Research. The programme will be concerned with the physical, chemical, biological and medical effects of radiations, particularly ionizing radiations. Research workers in these fields will be invited to proffer original papers and reports of new experimental work. A brochure will be available in April 1961. Information may be obtained from Dr. Alma Howard, Secretary-General, The Second International Congress of Radiation Research, Mount Vernon Hospital, Northwood, Middlesex, England.

THE XIIITH INTERNATIONAL ORNITHOLOGICAL CONGRESS

The XIIIth International Ornithological Congress will be held at Cornell University, Ithaca, New York, from 17 to 21 June 1962, under the presidentship of Prof. Ernst Mayr. General Secretary, Prof. C. G. Sibley, Fernow Hall, Cornell University, Ithaca, New York, U. S. A.

NOTICE

Owing to the rise in the cost of material and printing charges we are compelled to make a slight increase in the price of the Journal with effect from 1962. The new rates are as follows:

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MANAGING EDITOR,
J. Anim. Morph. Physiol.

STUDIES ON THE EXTERNAL MORPHOLOGY OF *PEREGRINUS MAIDIS* (ASHMEAD) (HOMOPTERA, FULGOROIDEA, ARAEOPIDAE = DELPHACIDAE)

Part I, Head capsule and mouth parts

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Department of Zoology, Government College, Ajmer, India

Morphological studies on representative types of the superfamily Fulgoroidea have not been adequately carried out particularly that of Araeopidae, though a few workers like Tower (1914), Myers (1928), Qadri and Aziz (1950) and Akbar (1957) have studied in detail the morphology of certain hemipterous bugs, hardly any significant work has been done on the araeopids. The present work on the external morphology of the head capsule and mouth parts of an araeopid, *Peregrinus maidis* (Ashmead) is therefore intended to fill a part of this gap.

Peregrinus maidis (*pundaluyoa simplicia* Distant), the corn plant hopper, was recorded for the first time by Ashmead in 1890. These insects are known vectors of sugarcane and maize mosaics. At times, they occur in large numbers as a distinct pest of maize. They are found in the tropical and warm temperate regions of the world wherever maize is grown.

Material and Method

The insects were initially collected in light traps from Coimbatore in south India during the months of October and November, 1959. Later, the authors could collect them locally. The heads were made transparent either by treating with cold concentrated potassium hydroxide solution for six to eight hours at room temperature or by boiling in a dilute solution of the same (5%) for about ten minutes. Subsequently the sclerites were stained in 1% solution of picro-indigo carmine and studied under a stereoscopic binocular microscope.

Description and Discussion

Head Capsule (Figs. 1, 2, 3 & 4)

The head of *Peregrinus maidis* is conical in shape. The mouth parts are of the opisthognathous type. The most conspicuous surface structures of the head are the large, strongly convex, reniform, compound eyes, which

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occupy a considerable area on the lateral sides of the head. The area between the anterolateral margins of the eyes represents the face. There are a number of carinae on the surface of the head capsule, which are of systematic importance. The clypeus is tricarinate, with one median and two lateral carinae. They join with the corresponding carinae of the facial region which is five carinate. The median one is the stoutest, and there is a gradual gradation in thickness from the middle one to the outer ones. The outer carinae terminate at the base of the antennae, whereas the inner ones continue to the top of the head apposing the compound eyes. The median carina bifurcates at about half of its length and continues posteriorly on the top of the head. There they diverge widely to join the lateral carinae of that region, which pass apposing the compound eyes. Posteriorly there is a small carina joining the two mediolateral carinae. From the middle of this carina extends a faint mediolateral carina upto the occipital suture.

Fig. 1 Cephalic view of head.
Fig. 2 Caudal view of head capsule.
Fig. 3 A. Lateral view of head capsule.
B. Top of head (boundry of vertex marked in dots).
Fig. 4 Tentorium.
Fig. 5 Dorsal view of labium.
Fig. 6 A. Dorsal view of apical segment of labium (higly magnified).
B. Mandibular and maxillary stylets in transverse section (highly magnified).
Fig. 7 Dorsal view of hypopharynx showing lora.
Fig. 8 Ventral view of hypopharynx showing its relation with salivary syringe.
Fig. 9 Mandibular and maxillar stylets and salivary syringe in relation with the head capsule.
Fig. 10 A. Mandibular stylet.
B. Maxillary stylet.

ac—anteclypeus; al—apical lobe of labium; ap—apodeme at the lateral margin of the fourth labial segment; ata—anterior tentorial arm; bt—body of tentorium; ce—compound eye; da—dorsal tentorial arm; ec—ejection canal; er—epistomal ridge; es—epistomal suture; fl—flagellum; fm—foramen magnum; fr—frons; ge—gena; hps—hypostomal suture; hs—hypopharyngeal suspensorium; jad—junction of the anterior and dorsal arms; lb—labrum; lg—labial groove; lo—lateral ocellus; lor—lorum; lp—labial plate; lpa—labial plate apodeme; lpp—labial plate proce.s; mds—mandibular stylet; ml—mandibular lever; mp—mandibular plate; mph—median process of hypopharynx; mxl—maxillary lever; mxp—maxillary plate; mxs—maxillary stylet; mxss—maxillary stylets; oa—outer arm of mandibular stylet; oc—occiput; ocs—ocular sclerite; os—occipital suture; pal—process of labial apical lobe; pc—postclypeus; pe—pedical; pge—postgena; ple—pleurostomal suture; pn—piston; poc—postocciput; pos—postoccipital suture; pt—posterior tentorial arm; rj—ring joint; sc—scape; scl—suction canal; ss—salivary syringe; vph—ventral process of hypopharynx; vx—vertex

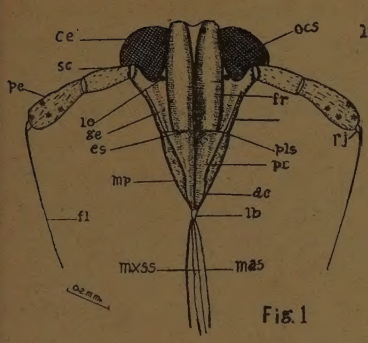
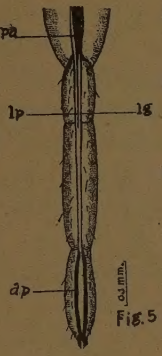


Fig. 1



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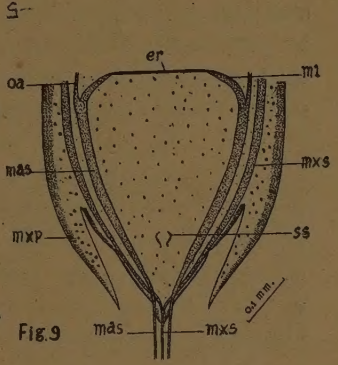
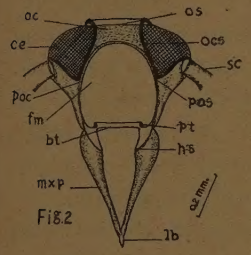


Fig. 9



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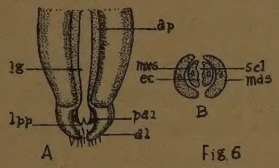

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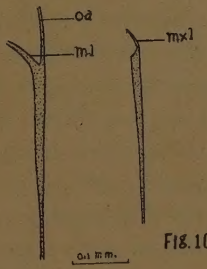

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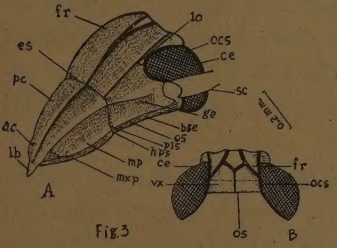

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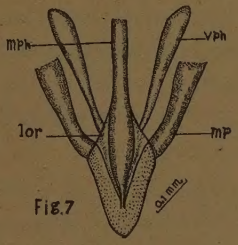

Fig. 7

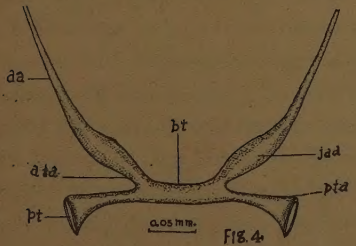

Fig. 4

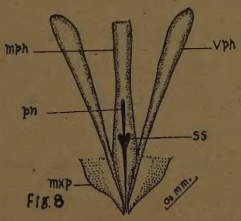

Fig. 8

The clypeus is a conical piece on the anterior side of the face. The anteclypeus and postclypeus are separated by a junction which is marked by two lateral constrictions. The clypeus is limited posteriorly by the epistomal suture bearing internally a well developed ridge. The epistomal suture is arched posteriorly and connects the pleurostomal suture of both the sides. The clypeus is bounded laterally by sutures which separate it from the lateral mandibular plates. The labrum is small, conical, less chitinised, freely hanging from the clypeus. There is no clypeolabral suture separating the clypeus from the labrum. The junction between the two is thinly chitinised. The labrum is strongly convex dorsally, and concave ventrally, forming a cover for the basal part of the labium. It extends upto the proximal portion of the third labial segment.

As a result of the absence of the ecdysial clevage line (formerly known as epicranial suture) the frons and vertex form a composite structure. The top of the head visible dorsally in profile has so far been considered as vertex and the remaining part as frons in araeopids. Snodgrass (1947) advocated a study of facial muscles for determining the correct homologies of the surface parts of the insect cranium. The origin of the labial muscles usually marks the posterior boundary of the frons. Accordingly, in *Peregrinus maidis* the vertex restricts itself as a rectangular part behind the union of the mediolateral and lateral carinae. Thus the hitherto considered vertex includes both the vertex as well as a part of the frons. The combined frontovertex region extends from the epistomal suture to the occipital suture.

The mandibular plates limit the lateral margins of the clypeus. Each plate is bounded on all the sides by the sutures. Posteriorly, it is separated from the gena by the pleurostomal suture, which runs obliquely from the lateral side to the front to join with the epistomal suture. The mandibular plate is analogous to the pleurostoma of a generalised insect. It has been termed by Myers (1928), Hamilton (1931), Butt (1943) and Snodgrass (1944) as ' lorum ' and by Weber (1933) as mandibular plate. As such the terms ' lora ' and mandibular plates become synonymous. Later Qadri and Aziz (1959) suggested that the term lora should be used for the two vertical plates on either side of the clypeus from the inner margin of the mandibular plates. These plates are separated from the mandibular plates by distinct sutures and are dorsolateral expansions of the hypopharynx.

The maxillary plate is a small, pointed, convex sclerite, extending

from the foramen magnum to the anterior tip of the clypeus. It lies at the lateral side of the mandibular plate in close approximation and is separated by a distinct vertical suture. Posteriorly, the maxillary plate is separated from the postocciput by a faint hypostomal suture. It bears a narrow strip at its inner side, the lever, which connects it with the base of the maxillary stylet.

The frons continues laterally with the parietal region. The parietal region is bounded posteriorly by the occipital suture. It bears compound eyes, antennae and lateral ocelli. Each compound eye is encircled by well developed ocular sclerites. The antenna is situated immediately behind the compound eye. It consists of three parts; the scape, the pedicel and the flagellum. The basal rim of the scape is lodged in the circular antennal socket. The antennal suture, which surrounds the antennal socket, is inconspicuous. On either side, at the dorsal and ventral margins, the basal rim of the scape is pointed and more chitinised than the remaining parts. The scape is covered with minute hairs. Similar to the basal projections of the scape, there are two at the base of the pedicel. This brings a dicondylic articulation with the scape. The pedicel* is considerably longer than the scape and is beset with a number of sensoria. They are of two types, minute hair-like and plate-like. There is a small oval structure, the ring joint, attached to the anterior region of the pedicel. The latter is continued as the flagellum. The lateral ocellus is situated near the junction, where the lateral carina apposes the compound eye. The median ocellus is absent. The part of the parietal region below the compound eye represents the gena. Its lower limit is marked by the pleurostomal suture. Caudal to the gena is the postgena, surrounded by the postoccipital and hypostomal sutures, similar to the generalised insect cranium.

The occipital suture is distinct and is situated dorsocaudad to the postoccipital suture. It is visible as a transverse suture between the compound eyes. It is then extending from anterior side of the compound eyes to the caudal part of the pleurostomal suture. This incomplete nature of the suture is brought about by the enlargement of the compound eyes. The area between the occipital suture and the postoccipital suture is the

* According to Muir's key (1915) for generic determination *Peregrinus* has the scape lesser than half the length of pedicel. However, in these specimens the scape is more than half the length of the pedicel. These specimens agree with the specimens of *Pundaluoya simplicia* identified in the National Pusa Collection, New Delhi.

occiput. The submarginal suture running along the dorsolateral margin of the foramen magnum represents the postoccipital suture. It terminates laterally at the posterior tentorial pits. Internally, the suture bears a thin postoccipital ridge. The postoccipital suture separates a narrow membranous strip along the dorsal and lateral margins of the foramen magnum, the postocciput. The neck membrane is attached to the postocciput.

In *Peregrinus maidis* there is a well developed transverse bar across the foramen magnum, representing the body of the tentorium. The posterior pair of tentorial arms takes origin from the posterior tentorial pits and run transversely to join the body of the tentorium. From the body of the tentorium arises a pair of diverging processes extending to the antennal bases. Each process exhibits a swelling (Fig. 4, jad) immediately after its origin. The swelling represents the place of the union of the dorsal tentorial arm with the anterior tentorial arm. As such, that part of the process between the swelling and the antennal base represents the dorsal tentorial arm, while that between the swelling and the body of the tentorium, the anterior tentorial arm. The part of the anterior tentorial arm between the union of the dorsal and anterior arms, and the anterior tentorial pit (the anterior tentorial pit which is theoretically present either in the epistomal or subgenal suture is not at all discernible in *Peregrinus maidis*) has been completely atrophied. The function of the anterior tentorial arm is to strengthen the facial region of insects, which is taken up in *Peregrinus maidis* by a well developed, highly sclerotised epistomal ridge. Consequently, the anterior arms have lost their function and have atrophied. Such a condition can be derived from *Pyrilla perpusilla* (Qadri and Aziz, 1950), a member of Fulgoroidea, in which the part of the anterior arm towards the anterior tentorial pit is becoming slender and shows a tendency of reduction. Taking this as an intermediate stage the reduction in the anterior arm of *Peregrinus maidis* can be easily explained.

Mouth Parts (Figs. 5, 6, 7, 8, 9 & 10)

(a) *The Labium :* The labium is a tubular, four jointed structure with a shallow groove along its dorsal surface. When not in use it is kept abreast with the ventral surface of the head and thorax with the tip reaching beyond the procoxae. The first segment is less chitinised than the remaining three. It is attached to the maxillary plate by the labiomaxillary membrane. The groove of the labium is floored by a chitinous plate, the labial plate. It is produced anteriorly into an apodeme, the labial apodeme of Myers (1928). The labial plate becomes thin posteriorly

while its lateral sides are more chitinised. The second and third segments are similar in structure to that of the first. The third segment is the longest. In the fourth segment the lateral walls are provided with two apodemes. When the labial muscles act, the lateral margins come closer to each other with the result that a tube is formed with the stylets included in it. The labial apex (Fig. 6, A) is deeply emarginated and its sides develop into apical lobes (al). A short process is borne on the inner side of the each lobe (pal). The labial plate at the tip bifurcates into two sclerotised processes (lpp). This structure lies mesoventral to form the lower side of a ring round the stylets. The upper side of the ring is completed by the processes of the labial apical lobes, which comes near and near when the labial muscles contract. The tip of labium bears sensory hairs.

(b) *The Hypopharynx* : The hypopharynx has undergone profound modifications from the generalised type and is fused with the salivary syringe. It is a sclerotised pad-like structure, with a cylindrical central mass. The body of the hypopharynx consists of a small median lobe with a pair of laterodorsal, a pair of ventral and a single median dorsal process. The paired laterodorsal processes arise from the dorsal surface of the central mass of the hypopharynx and run in a laterodorsal direction to end on the dorsal area of the respective mandibular plates at the junction between the ante and postclypeus. These are the lora. They together with the median lobe support the sucking pump. The paired ventral processes arise laterally from the central mass of the hypopharynx and extend posteriorly toward the foramen magnum. They are flat, thin plates which gradually broaden distally. Posteriorly they are fused with the posterior tentorial arms. At their bases they are supported by much chitinised small, almost conical sclerites, the hypopharyngeal suspensoria. The ventral processes give attachment to the muscles of the salivary syringe. The median dorsal process arises from the ventral wall of the median hypopharyngeal lobe. It runs medially towards the posterior region of the head as a sclerotised hollow trough. Posteriorly, it extends beyond the maxillary lever. The salivary syringe is in the form of a cup-shaped, highly chitinised, cavity on the median lobe of the hypopharynx. The cavity is continued anteriorly into a narrow exit duct which runs through the median lobe to terminate at the ejection canal. In the posterior region of the cavity there is a thick process or piston which is provided with a handle. The handle exhibits a restricted movement during the pumping out of the saliva.

(c) *The Mandibular Stylets :* The mandibular and maxillary stylets are long, bristle-like, arising between the ectal wall of the hypopharynx and the ental aspect of the maxillary plates. The mandibular stylets are shorter than the maxillary stylets. They converge in the mesoanterior direction to pass below the lora. Their converging enables them to come closer to the maxillary stylets till the labial base is reached, where they are applied on the outer surface of the maxillary stylets. The inner side of the mandibular stylet is concave which rests against the outer convex surface of the maxillary stylets, but being shorter in length leaves the apex of the latter uncovered. Basally the mandibular stylet is thicker and it narrows down posteriorly. From the base it gives two arms posteriorly, an inner and an outer. The outer arm is in the form of a long apodeme and extends deep into the lateral wall of the head capsule. The inner arm is joined to a small sclerite, the lever, which runs transversely to join the lateral margin of the epistomal suture.

(d) *The Maxillary Stylets :* Each maxillary stylet runs along the inner face of the maxillary plate. They are thinner than the mandibular stylets. Their bases extend deep into the head than those of the mandibular stylets. Anteriorly, they converge and enter into the labial groove. Similar to the mandibular stylets the bases of the maxillary setae are thickened and gradually narrow distally. A sclerotised strip, the maxillary lever, is joined with the base of the maxillary seta. The other end of the lever is attached to the base of the maxillary plate. Unlike that of the mandibular stylet there is no arm supporting the base of the maxilla. The inner surface of each maxillary stylet develops a mid-longitudinal ridge (Fig. 6, B) which divides it into an upper and a lower continuous groove. These grooves in union with the corresponding grooves of the other stylet form an upper complete canal called the suction canal and a lower, called the ejection canal. The separation of the canal is completed by the apposition of the two ridges. The suction canal is wider than the ejection canal, while the latter conveys saliva from the salivary syringe during suction.

Summary

A detailed account of the morphology of the head capsule and mouth parts of an araeopid, *Peregrinus maidis*, is given.

1. The clypeus and the labrum are fused together.

2. The ecdysial clevage suture is absent and the frons and the vertex are fused together.

3. The area of vertex is restricted to a small rectangular region on the top of the head behind the junction, where the mediolateral carinae join with the lateral carinae.

4. The mandibular plate and the lorum are antonymous and the latter represents the laterodorsal process of the hypopharynx.

5. The antennae are conspicuous, with the pedicel considerably longer than the scape. The scape is provided with sensoria.

6. The occipital suture is interrupted in its course by the extension of the compound eyes. The postoccipital suture is distinct.

7. The anterior tentorial arm is reduced, whereas the posterior and dorsal arms are well developed.

8. The labium is four jointed.

9. The hypopharynx consists of a small, median lobe, with a pair of laterodorsal, a pair of ventral and a single median dorsal process.

10. By the apposition of the maxillary stylets the suction canal and the ejection canal are formed. The mandibular stylets are applied on the outer surface of the maxillary stylets.

Acknowledgments

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"GIANT" FIBRES IN THE CENTRAL NERVOUS SYSTEM OF SCORPION

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(One Plate)

IT has now been accepted by all that the giant fibres are true nervous structures. These are non-myelinated fibres that can conduct impulses at a faster rate than the fibres of smaller diameter. Much work has been done on the giant fibre system in several invertebrates. These structures are best known in annelids (Stough, 1926; Bullock, 1945a, b, 1948; and Nicol, 1947 and 1948 a, b), cephalopods (Young, 1934, 1936 a, b, c, 1939 and 1944), crustaceans (Johnson, 1924; Holmes, 1942 and Wiersma, 1947) and insects (Roeder, 1948; Power, 1948; Cook, 1951; Hughes, 1953; Satija, 1957; 1958 a, b, c, d; Pipa and Cook 1959)

Nicol (1948 b) in his article defined giant axons as nerve fibres in any species that are disproportionately greater in size than the other nerve fibres of the animal and a functional distinction, effected by widespread and synchronous or nearly synchronous muscular contractions (Young, 1944). It is in these meanings the term "giant fibre" is employed in the following discussion.

Except in insects and crustaceans, little is known of such fibres in the varied groups of arthropods, although several workers have dealt with the nervous system in such arthropods as the arachnids. During the course of an extensive and detailed study of the nervous system of arachnids, certain relatively large fibres were noticed in the scorpion and the present paper gives an account of such fibres.

Materials and Methods

Two species of Scorpion (*Heterometrus swammerdami* and *H. fulvipes*) which are locally available were used. The animal was dissected either in the living condition or after chloroforming. As soon as the brain and the nerve cord was exposed, the dissection tray was filled with Bouin's fixa-

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tive. The further process of cleaning and removing the brain and the nerve cord was carried on while the specimen was immersed in the fixative. The blood vessel over the nerve cord was removed so as to prevent torsion during the process of dehydration. The entire nerve cord from the 1st to the 7th ganglion was fastened to a straight rod and removed from the animal and kept in fresh Bouin's fluid for 24 hours. The brain also, after removal from the animal, was kept in fresh Bouin's for 24 hours. The nerve cord, after fixation was cut into seven pieces corresponding to the seven ganglia. Sections were cut in paraffin from 10 to 20 μ in thickness and the connectives were stained by Holmes (1952) silver technique while the brain and ganglia were stained with Palmgren (1948) silver technique. Drawings were made using the microprojection apparatus.

Observations

The giant fibres in the ventral nerve cord :

The ventral nerve cord of the scorpion which is longest, with the largest number of free ganalia amongst arachnids, appears to possess only the unicellular type of giant fibres. These giant fibres have their origin in two ways : from the free abdominal ganglia and from the sub-oesophageal ganglion.

With the exception of the 7th ganglion, the rest of the ganglia have 30 to 40 large cells measuring 78·5μ in diameter. The 7th ganglion which has two fused ganglionic masses representing the 4th and 5th metasomatic segments has a larger number (50 to 60) of these large neurocytes. In this the largest neurocyte is 110μ in diameter. The exact position of these large neurocytes differs from one ganglion to another, depending upon the origin of the lateral segmental nerves. In each ganglion they are mainly divided into two groups situated one on either side of the central neuropile and towards the ventro-lateral part. From each side, the axons ascend up towards the dorsal side, move towards the centre, give off several collaterals, bend back and enter the segmental nerve on the same side. Thus in this region of the ganglion the middle part is formed of the collaterals coming from each half of the giant fibres. Some of them may even pass into the opposite segmental nerves. Through this dorsal and collateral part of the ganglion the mid-dorsal through tract passes, containing giant fibres.

The seven free abdominal ganglia are traversed by seven pairs of thorough fibre tracts (Fig. 1). These seven tracts are connected with the

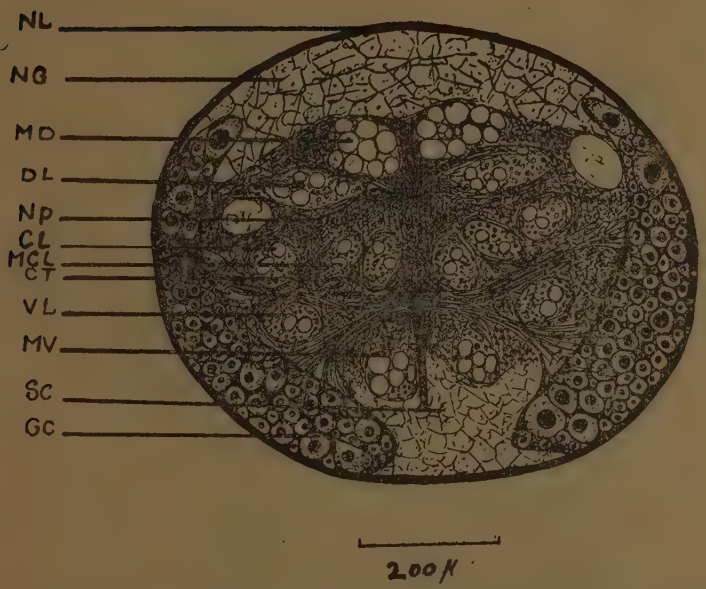

Fig. 1: T. S. of the second abdominal ganglion of scorpion (*camera lucida* drawing). NL: Neurilemma; NG: Neuroglia; MD: Mid-dorsal tract; DL: Dorso-lateral tract; NP: Neuropile; CL: Centro lateral tract; MCL: Mid-centro-lateral tract; CT: Central; tract; VL: Ventro-lateral tract; MV: Mid-ventral tract; SC: Ventral associative centre; GC: Ganglion cells.

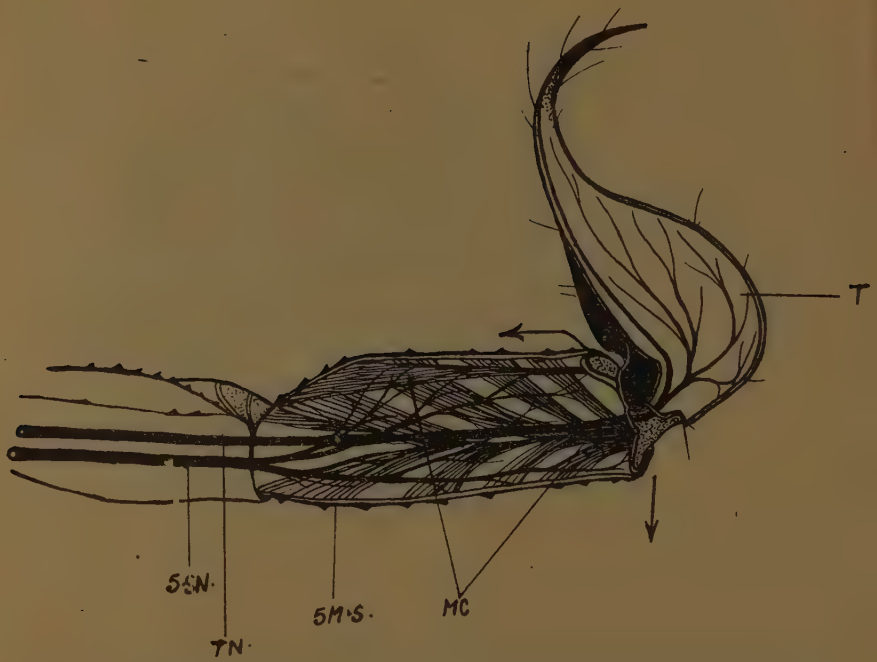

Fig. 2 : Innervation of the muscles of the 5th metasomatic segment and the sting (Diagramatic)
5. SN : 5th, metasomatic segmental nerve; TN : Telsonic nerve; 5. MS : 5th metasomatic segment; MC : Muscles responsible for the movement of the sting; T : Telson.

seven major tracts of the suboesophageal ganglion. Some of these tracts disappear in the 7th ganglion. The others pass through all the ganglia and straight enter into the 5th metasomatic segmental nerve.

Each tract appears to have a set of "giant" fibres. In the mid-dorsal tract there are 9 large fibres. The cell bodies of these fibres are in the suboesophageal ganglion and the axons pass straight through all the six ganglia. In the 7th ganglion a few of these fibres end near the 4th metasomatic segmental giant fibres. The rest of them end in the vicinity of the 5th metasomatic segmental giant fibres. A few of them enter deep into the neuropile.

In the dorsolateral tract there are 5 fibres. The large fibres in the three central tracts appear to increase in their number from the 1st to the

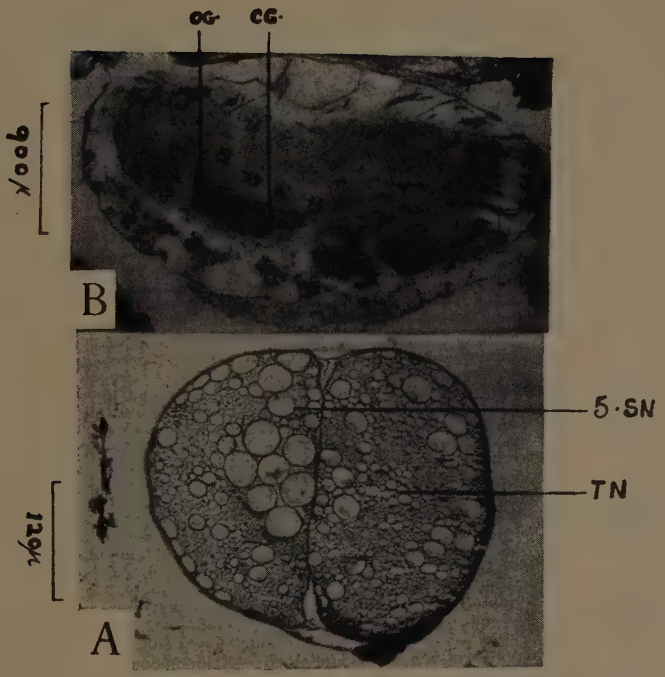

Plate I. A. T. S. of the 5th metasomatic segmental and telsonic nerves showing the giant fibres. Section 14 μ thick, stained with Holmes silver technique.

5. SN : 5th metasomatic segmental nerve.

TN : Telsonic nerve.

B. Sagittal section passing through the central ganglion of the sub-oesophageal ganglion, showing the origin of the giant fibres.

Section 25 μ thick, stained with Palmgren silver technique

OG : Origin of giant fibres.

CG : Central ganglion

last ganglion. In the 1st ganglion they are five in number and in the anterior part of the 7th ganglion they increase to ten. It is not quite clear whether this increase is due to the addition of larger fibres from the abdominal ganglia or due to an increase in the diameter of some other fibres in these tracts. Some of the fibres within the 7th ganglion show an increase in their diameter as they move out of the ganglion. Likewise in the ventrolateral tract also there is an increase in the number of larger fibres from the anterior to the posterior, there being two in the 1st ganglion and five in the last ganglion. The large fibres in these tracts become more and more conspicuous in the last ganglion.

In the mid-ventral tract there is an increase in the number of giant fibres anteriorwards from the 7th ganglion to the 1st. In the 7th ganglion there is only one pair and in the 2nd free ganglion anteriorly there are six pairs. Thus there is an increase of fibres from posterior towards the anterior side of the nerve cord. The cell bodies of these fibres have not been observed. Out of the six large fibres four are in one bundle. One pair from this bundle disappears near the dorsal side of the first pectinal mass in the suboesophageal ganglion. The other three fibres move forwards to the vicinity of the central ganglia and enter the longitudinal fibre tract "A" of the suboesophageal ganglion (Sasirababu, 1961). The remaining two large fibres anastamose near the dorsal side of the second pectinal mass. Thus all the six large fibres from the ventral nerve cord enter the so called "ventral associative region" of the suboesophageal ganglion which is supposed to be the sensory region (Sasirababu, 1961).

In the nerve cord the size of the large fibres is 15.7μ. But the fibres that innervate the 5th segment are the largest "giant" fibres measuring 39.2μ in diameter. These big fibres innervate the muscles of the 5th segment which are responsible for the various movements of the sting. The sting is also innervated by large fibres with a diameter 25.7 μ.

Giant fibres in the sub-oesophageal ganglion :

Besides the large fibres in the ventral nerve cord described above, several large fibres measuring about 31μ in diameter arise in the central part of the sub-oesophageal ganglion and enter the pedipalpal nerve. These giant fibres appear to be syncytial in origin. The cell bodies of these fibres could not be traced. Certain small cell-like structures are seen in the central ganglion and these give off fine fibres. Whether these are the sources of the giant fibres could not be established. Similar fibres

of smaller diameter (about 25μ) also enter the leg nerves, arising from the more lateral parts of the sub-oesophageal ganglion. A fuller description of this system will be given elsewhere (Sasirababu, 1961).

Discussion

The syncytial type is more common in several invertebrates (annelids, crustaceans, insects and cephalopods). The unicellular type is less common and has been described only in a few forms (nemerteans and cestode worms, in numerous polychaetes, in balanoglossids, and in lower vertebrates). In scorpion on the other hand the unicellular "giant" fibres are greater in their number, present not only in the sub-oesophageal ganglion but also in the ganglia of the ventral nerve cord. They innervate the pedipalps, four pairs of walking legs and each subsequent segment, from the ganglia of the ventral nerve cord. The large fibres arising in the sub oesophageal ganglion, which are likely to be multicellular are less in number and innervate only the pedipalps, four pairs of walking legs and a few pairs may pass into the ventral nerve cord.

The largest "giant" fibres measuring 39.2μ in diameter appear to have their origin from the neurocytes of the 7th ganglion innervating the 5th metasomatic segment. The muscles of this segment are mainly responsible for the various movements of the sting (Fig. 2). The next largest fibres go to the pedipalps, the sting and the four pairs of ambulatory legs. Scorpions when they are alert (fighting, capturing food, facing danger) have a characteristic posture : raising the pedipalps, bringing the tail over the prosoma and standing on the legs. The relation between these three can be correlated with the pattern of "giant" fibre arrangement. The central paired bodies of the sub-oesophageal ganglion are connected to the pedipalps anteriorly and with the ventral nerve cord posteriorly. The four pairs of ambulatory legs have their transverse commissures spread over the central paired bodies. Thus the important organs are connected to each other in an integrated manner through the "giant" fibre system. Physiological studies now in progress are designed towards an analysis of such a system of fibres.

Summary

In the central nervous system of scorpions there are two types of giant fibres (a) unicellular type, present in the sub-oesophageal mass as well as in the seven free abdominal ganglia and (b) large fibres arising

only in the sub-oesophageal ganglion whose origin is not clear, but which may be "multicellular" in origin.

The giant fibres in the ventral nerve cord arise either from the sub-oesophageal ganglion or from the free abdominal ganglia.

There are seven pairs of fibre tracts running through all the six abdominal ganglia. In the seventh ganglion two of them disappear and the rest pass through into the sting and the fifth metasomatic segmental nerves.

The largest giant fibres appear to arise in the seventh ganglion and innervate the muscles of the fifth metasomatic segment (39.2 μ). A correlation between the pattern of arrangement of the "giant" fibres and the defensive and offensive posture of the scorpion is suggested.

Acknowledgement

I am deeply indebted to Professor Kandula Pampapathi Rao, Professor and Head of the Department of Zoology, for suggesting this problem and for his constant guidance throughout the course of this investigation.

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IS NOT THE SO-CALLED LYMPH GLAND OF THE SCORPION ALSO AN ENDOCRINE ORGAN?

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(One Plate)

A glandular organ associated with the nerve cord and the supraneural artery has been described by Awati and Tembe (1956) in the scorpion *Buthus tamulus*. According to them the glandular organ is made up of a band of tissue with eleven nodules distributed along its length. It is said to extend from the diapharagm to the 13th segment, and in its disposition binds the nerve cord with the supraneural artery. The gland has been described to consist of lymphocytes, leucocytes, acidophils, basophils and certain giant cells. While reviewing the previous literature, they found support for their observation in a paper by Pavlosky who seems to have found in the gland blood cells in various stages of development and also otlers worn out, awaiting possibly destruction. In the light of their observations and those of earlier workers they concluded that the gland functioned as a lymph gland similar to the spleen.

During the course of routine dissections the so-called lymph gland in the scorpion *Palamnaeus fulvipes* attracted our attention. After examining the structure under the microscope it was suspected that it was more than a lymph gland. A detailed histological and histochemical examination was called for. The organ was therefore dissected out and immediately fixed in Bouin's fluid for studying its general histological structure and others were treated with formol dichromate solution for testing chromaffin reaction (Coupland, 1954). Paraffin sections of Bouin's fluid fixed material were prepared and stained with Delafield's haematoxylin and counter stained with eosin. These were mounted in canada balsam. Those treated with formoldichromate were subjected to chromaffin reaction with dilute Giemsa (1 ml to 20 ml phosphate buffer pH 7.2) for 24 hours. After having been differentiated in 0.5 per cent glacial acetic acid, the sections were passed through 90 per cent and 100 per cent alcohol to xylene and mounted in damar.

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Observations

The glandular organ in *Palamnaeus fulvipes* is not externally divided into nodules so as to make it appear distinct from the so-called supraneural artery as in *Buthus tamulus* noted by Awati and Tembe. In other words in the scorpion *Palamnaeus fulvipes* the gland and the so-called artery are one and the same structure. The nerve cord and the gland are very closely approximated, though they can be easily separated from one another. As nerves issue from the ventral nerve cord a branch of the gland accompanies each of them. The gland is thick at the prosoma and mesosoma regions but tapers somewhat before joining the ring vessel around the oesophagus. Posteriorly also the gland tapers as it proceeds to the metasoma. There is a spaceous lumen at the thick part of the gland and this lumen is occupied by a colloidal translucent secretion (fig. 1). At the tapering anterior and posterior regions, the lumen is very narrow and in certain regions seems to be practically non-existing. There is no lumen found at least in paraffin sections in the branches of the gland Occasionally the lumen in the thick region of the gland is devoid of the secretion and will be only occupied by a thin fluid or lymph. Again in certain sections free cells might be found in the colloidal secretion. Presumably they got detached from the gland and got imbedded in the secretion (Figs. 1 and 2).

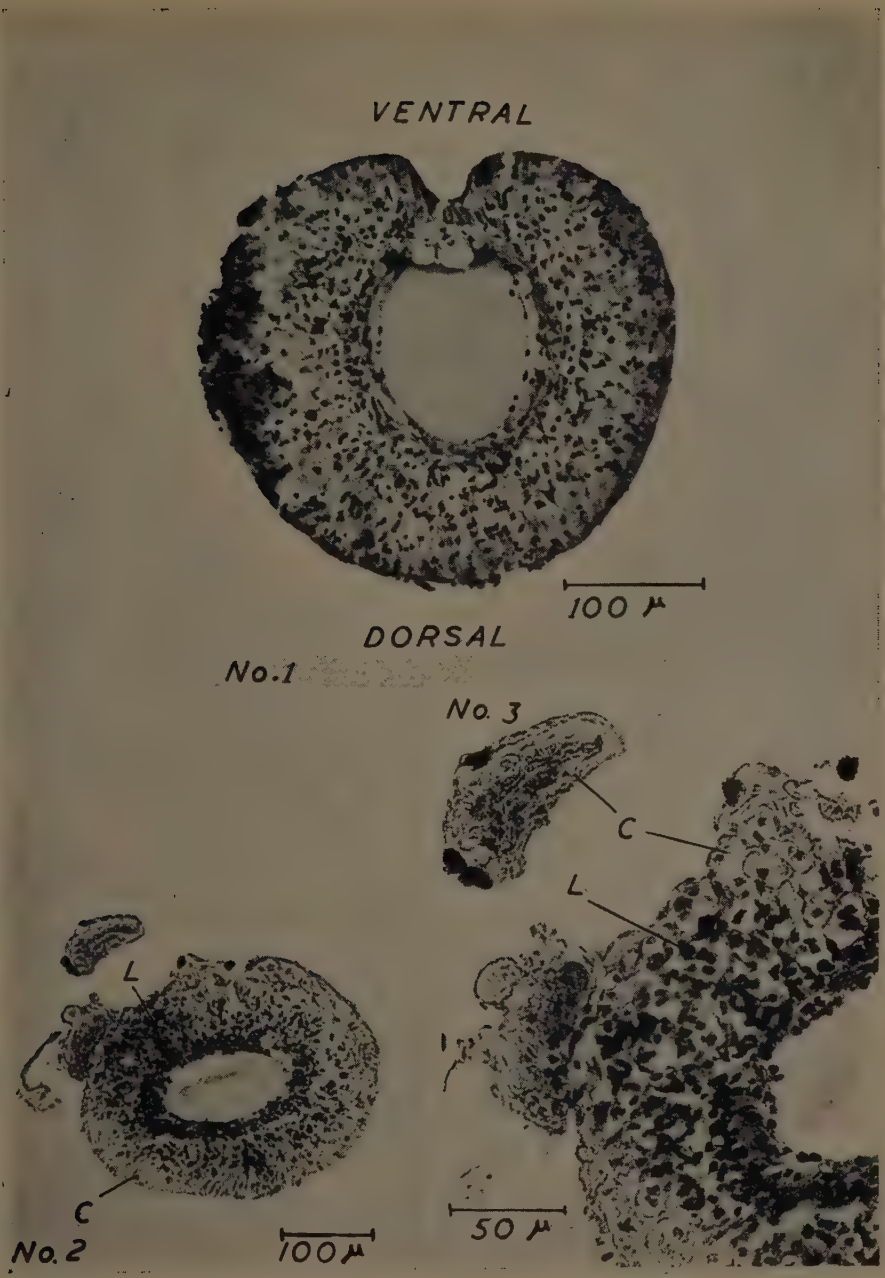
The cortical region of the gland is made up of large cells with copious amount of cytoplasm. There is an inner lining layer of epithelial cells which get stained pink with eosin. The other cells with large nuclei are stained purple and they give the appearance of lymphocytes.

The peripheral cells showed light green positive chromaffin reaction demonstrating chromaffin granules. The chromaffin reaction is also met with in the branches of the gland. In the branches the layers of lymphocytes are not in evidence (Figs. 2 and 3).

Further work on the nature of the gland is called for and it is in progress.

Discussion

The homology of the gland is difficult to explain. From the connection it has with the blood-vascular system anteriorly, there is some ground for taking it for a blood vessel as others have done. From the nature of its cells, it certainly is a gland and not a blood vessel. Its supraneural position, if it is homologous with a blood vessel is strange, since in that location only a subneural vessel could be expected. Lymph glands in that



J.C.G., D.J., A.W. & O.G.B.

Plate I

Fig. 1 T. S. of the gland showing the general histology and the colloidal content of the lumen.

Figs. 2 & 3 T. S. of the gland and its branches treated for the chromaffin reaction.

C. Cells showing positive chromaffin reaction.

L. Cells resembling lymphocytes with large deeply stained nuclei.

position have not been reported in arthropods. Subsequently a glandular organ of similar nature has been noted in the whip scorpion *Telephonus sp.* In that animal the ventral nerve cord is reduced and posteriorly the branch of the gland has been found to run ventral to the pair of nerves proceeding back from the large ganglion. On the other hand its histological structure is definitely that of a gland. If it is of blood vessel nature, some indication of smooth muscles on its walls is likely. No trace of any muscle cells is seen. The inner lining cells where a lumen is present are epithelial and such may be found both in a blood vessel and in a gland. Again if it is a blood vessel, it should taper only posteriorly and not anteriorly. In this case the lumen is conspicuous where the gland is thick and where it tapers and in the branches a conspicuous lumen is not in evidence. There is therefore no convincing evidence to homologise the gland with a blood vessel.

As regards its probable functions, the nature of the chromaffin secretion in the peripheral cells indicates that the gland is of endocrine nature. The appearance of the colloidal secretion seen in the lumen is comparable to the one in the thyroid. Some of the cells in the gland might be lymphocytes though they appear to be larger than the lymphocytes present in the blood. Extensive investigation is necessary to establish the nature of the endocrine function the gland performs.

Summary

In the so-called lymph gland of the scorpion, chromaffin reaction in the cytoplasm of certain cells have been demonstrated. It is likely therefore that the colloidal secretion met with in the lumen of the gland is of endocrine function.

Acknowledgements

We are deeply indebted to Professor C. J. George, formerly Hon. Professor of Zoology, M. S. University of Baroda and now Head of the Post-graduate Department of Zoology, American College, Madurai, at whose instance this work was undertaken, for his suggestions. We are also grateful to Professor V. B. Tembe for kindly identifying the scorpion.

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EFFECT OF GEOGRAPHICAL LOCATION, SEX OF THE CALF AND PARITY ON THE GESTATION PERIOD OF CERTAIN INDIAN CATTLE BREEDS

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IN India the existence of a wide variety of breeds, differing climatological conditions, and widely varying animal husbandry practices offer an unique opportunity for studying the effect of environment, breed, sex of calf and chronological order of gestation (parity) on a reproductive physiological process like the gestation period in cattle. Such a study carried out on five Indian breeds is reported in the present paper.

Material

The data utilised for the study came from individual farms located in different parts of the Indian Union and territories now in Pakistan. The basic data was abstracted from the records maintained by the farms. Information on the number of farms visited, number of cows included and the total number of gestations examined are presented in Table I. Obviously, for a study of this type it was necessary to have not only more than one farm where a given breed is located, but also sufficient number of records per farm and per gestation at each of the farms. This restricted the breeds to only five. They were *Sahiwal*, *Tharparker*, *Sindhi*, *Hariana* and *Kankrej*. Further, due to certain practical difficulties encountered in typing the gestations for normality, a subject considered separately in another paper, for the purpose of this study gestation lengths lying below 262 days and above 302 days were omitted. Such an arbitrary procedure will no doubt affect the standard deviations but not the means. It was on the mean values alone that our present study has been based and as such any influence which the selection of data might have on the conclusions drawn may be taken as negligible. In addition, gestations known to be definitely abnormal such as premature births, or still births, or those leading to twin or multiple births were also excluded.

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TABLE I

Details of basic data

Name of breed	Number of farms	Total number of cows	Total number of gestations
Sahiwal	7	1,746	5,052
Tharparker	4	691	2,381
Sindhi	6	723	2,503
Hariana	4	818	1,875
Kankrej	3	452	1,265
Total	24	4,430	13,076

Results

(i) *Sahiwal*

The mean gestation period found at the different farms are presented in Table II. For each farm, the gestation period for male and female births along with the overall gestation period are given separately. The number of gestations averaged is also given separately in brackets along with the means.

TABLE II

Mean gestation period (Sahiwal)

Sr. No.	Name of the farm	Average gestation period (in days) Male Births	Female births	Overall births
1	Agricultural College, Lyallpur	292.07 (183)	289.96 (228)	290 90 (411)
2	Government Cattle Farm, Lakhimpur-Kheri.	288.14 (129)	286.24 (131)	287.18 (260)
3	Indian Agricultural Research Institute, New Delhi.	288.20 (609)	285.97 (517)	287.17 (1,126)
4	Government Experimental Farm, Kankee.	288.76 (160)	284.55 (151)	286.71 (311)
5	Jehangirabad Farm.	286.01 (972)	284.59 (.976)	285.30 (1,948)
6	Agricultural College, Kanpur.	284.66 (134)	284.05 (134)	284.36 (268)
7	Montgomery Farm.	281.68 (406)	281.62 (322)	281.66 (728)
	Average (Total)	286.48 (2,593)	285.05 (2,459)	285.78 (5,052)

It is observed from Table II that male calves were carried for a longer period than female calves. Wide variation in average length of gestation period could also be seen from farm to farm. The highest was 295.90 days (Lyalpur) and the lowest was 281.66 days (Montgomery). The large variation appears to suggest admixture with other breeds. For example, Oliver (1938) mentions that large number of people from Rajputana and Kathiawar with their cattle at one time came into the area of the *Sahiwals* and that Gir blood introduced at that time still persists as evidenced from certain coat colour patterns. This would account for the upper limits, the lower limits could have been influenced by the introduction of foreign blood as for example the *Holstein-Friesians* by the Military authorities. Summary of detailed analysis of variance undertaken is presented in Table III. In the analysis, data from Lakhimpur-Kheri had been omitted since the data was confined to the first few gestations only.

By far the largest variation in gestation period in *Sahiwals* was due to farms. This meant that the genetic constitutions of samples of *Sahiwals* (as far as gestation period was concerned) maintained and reared at the different farms were markedly different. Obviously, this difference could not be due to the geographical location, since cows stationed at Montgomery and Lyalpur with practically like climatic conditions had significantly different average gestation periods. It is more reasonable to assume that the difference is due to inherent genetic differences. In addition, variation in average gestation period due to sex of the calf carried and interactions "sex × farms" and "sex × gestations" were statistically significant. Variation due to "parity" or order of gestation was not marked.

TABLE III

Analysis of variance (Sahiwal)

Source of variation	D. F.	M. S.	Variance components
Between Farms	5	160.97**	8.727
Between gestations	8	4.83	0.080
Interaction			
Farms × Gestations	40	3.87	0.690
Between sexes	1	26.42**	0.280
Interaction			
Sex × Farms	5	8.21*	0.636
Sex × Gestations	8	6.57**	0.513
Sex × Farms × Gestations (Error)	40	2.49	2.490

* Significant at 5% level ** Significant at 1% level

(ii) *Tharparker*

Summary of the mean gestation period found at the different farms is given in Table IV. The number of observations on which the mean values were based are given in brackets after the means.

TABLE IV

Mean gestation period (Tharparkar)

Sr. No.	Name of the Farm	Average gestation period (in days) Male births	Female births	Overall births
1	Indian Agricultural Research Institute, Karnal.	289.83 (556)	287.44 (535)	288.66 (1,091)
2	Government Farm, Kankee.	290.16 (126)	287.20 (137)	288.61 (263)
3	Livestock Research Station, Sakrand.	286.92 (39)	286.15 (41)	286.53 (80)
4	Government Farm, Patna.	287.32 (470)	285.30 (477)	286.30 (947)
	Average (Total)	286.87 (1,191)	286.62 (1,190)	287.75 (2,381)

The *Tharparkers* at Karnal had the highest and those at Sakrand, the lowest average gestation periods. The Karnal and Kankee animals had about the same order of gestation lengths. Similarly, the Patna and Sakrand *Tharparkers* had like level of mean gestation period. As in *Sahiwals*, it was noticed in case of *Tharparkers* also that the male gestations lasted a little longer than female ones. Omitting the Sarkrand data, which was scanty, detailed analysis of variance of gestation lengths was carried out. The results are summarised in Table V.

TABLE V

Analysis of variance (Tharparker)

Source of variation	D. F.	M. S.	Variance components
Between farms	2	31.39**	1.304
Between gestations	9	2.62	−0.448
Interaction			
Farms × gestations	18	5.31*	1.585
Between sexes	1	94.52**	3.137
Interaction			
Sex × Farms	2	1.10	−0.104
Sex × Gestations	9	1.45	−0.230
Sex × Farms × Gestations (Error)	18	2.14	2.140

* Significant at 5% level ** Significant at 1% level

From Table V, it would be seen that the largest contribution tot he observed variance in mean gestation lengths was due to sex of calf carried, followed by the interaction " Farm × gestations " and farms. All these were statistically significant. As in *Sahiwals* parity wielded no marked influence on mean gestation lengths. The other interactions such as " Sex × farms ", " Sex × gestations " were of a negligible order. Williamson (1947) had observed that *Tharparker* redesignated as " Thari " by him was not a homogenous breed and that it had been influenced by breeds like *Kankrej, Sindhi, Gir* and *Nagori.* He pointed out that in the Thari area, towards the western side, the influence of *Sindhi* was prominant; towards the northern and north-eastern side, the *Nagori* influence was dominant. In addition, a sprinkling of *Gir* influence was also in evidence. In view of this composite nature of the original breed, the farm to farm variation could be due to the differing sample of animals going to form the foundation animals of the herds, and later due to the varying amount of selection practiced in building up the herds.

(iii) *Sindhi*

In Table VI are presented the mean gestation periods of *Sindhi* cows observed at the different farms. The number of gestations averaged for the means are given in brackets.

Sindhi cows at Malir had the highest and those at Karachi, the lowest mean gestation periods. As in *Sahiwals* and *Tharparkers*, the bull calves were carried for a longer period than heifer calves. Results of detailed analysis in which Karachi figures had to be dropped being scanty, are summarised in Table VII.

TABLE VI

Mean gestation period (Sindhi)

Sl. No.	Name of the Farm	Average gestation period (in days) For male births	Female births	Overall births
1	Willingdon Farm, Malir	285.64 (152)	284.52 (111)	285.17 (263)
2	Livestock Research Station, Hosur	286.00 (149)	283.10 (213)	284.46 (362)
3	Agricultural Institute, Naini	285.56 (165)	282.09 (169)	283.79 (334)
4	Indian Dairy Research Institute, Bangalore	284.26 (379)	282.74 (379)	283.52 (758)
5	Agricultural College, Kirkee	284.02 (375)	281.45 (342)	282.79 (717)
6	Pinjrapole Society, Karachi	299.42 (36)	281.79 (33)	280.55 (69)
	Average (Total)	284.02 (1256)	281.45 (1247)	282.79 (2503)

TABLE VII

Analysis of Variance (Sindhi)

Source of Variation	D. F.	M. S.	Variance components
Between farms	4	14.83*	0.506
Between gestations	9	11.29	0.658
Interaction			
Farms × Gestations	36	4.71	−0.155
Between sexes	1	131.75**	2.592
Interaction			
Sex × Farms	4	5.80	0.078
Sex × Gestations	9	1.39	−0.726
Sex × Farms x Gestations (Error)	36	5.02	5.020

* Significant at 5% level

** Significant at 1% level

As in Tharparkers, the sex of the calf accounted for the highest variance in gestation period. Variance due to parity though fairly large did not approach the 5% level of significance. Once again the variance due to farms was statistical significant. None of the interactions approached the 5% level of significance.

(iv) *Hariana*

The mean gestation periods of Hariana cows at the different farms along with the number of gestations averaged are presented in table VIII.

TABLE VIII

Mean gestation period (Hariana)

Sl. No.	Name of the farm	Average gestation period (in days) Male births	Female births	Overall births
1	Indian Agricultural Research Institute, Karnal	291.39 (88)	289.70 (112)	290.44 (200)
2	Indian Veterinary Research Institute, Izatnagar	289.84 (174)	287.64 (192)	288.69 (366)
3	Government Cattle Breeding Farm, Bharari	288.30 (369)	286.62 (399)	287.43 (768)
4	Seed Demonstration Farm, Eilichpur	286.99 (269)	284.70 (272)	285.83 (541)
	Average (Total)	288.51 (900)	286.34 (975)	287.38 (1875)

The highest mean gestation period was observed in Karnal *Harianas* and the least in Ellichpur *Harianas*. As in the previous breeds, the bull calves were invariably carried for a longer period than heifer calves. It may be mentioned here that the Karnal and Izatnagar Harianas were practically the same, the latter being the transferred herd from Karnal subsequently augmented by purchases from the Hariana tract. The lowering of mean gestation period at Izatnagar, in a way reflects the systematic elimination of doubtful animals, and animals with poor *Hariana* characteristics by typical animals from Rohtak and Hissar region. In a way, this shows how the gestation periods could be shifted by a change in the make up and constitution of the herd. Results of detailed analysis of data are summarised in table IX.

TABLE IX

Analysis of variance (Hariana)

Source of Variation	D. F.	M. S.	Variance component
Between farms	3	52.33**	3.157
Between gestations	7	5.33*	0.439
Interaction			
Farms × gestations	21	1.82	−0.110
Between sexes	1	73.15**	2.182
Interaction			
Sexes × Farms	3	1.97	−0.009
Sexes × Gestations	7	3.40	0.340
Sexes × Farms × Gestations (Error)	21	2.04	2.040

* Significant at 5% level

** Significant at 1% level

In case of *Harianas*, the farm to farm variation accounted for the largest percentage of the total variance in mean gestation period, followed by sex of calf and parity. Unlike, other breeds studied, the variance due to parity came out to be significant at 5% level. One of the reasons for this result may be the genetic constitution of the herds considered and the mode of their subsequent breeding. The interactions studied were small and in no case approached the generally accepted level of statistical significance.

(v) *Kankrej*

Mean gestation periods at the different farms are presented in table X. In brackets the number of gestations averaged in calculating the means are also given.

TABLE X

Mean gestation period (Kankrej)

Sl. No.	Name of the farm	Average gestation period (in days) Male births	Female births	Overall births
1	N. C. Cattle Farm, Charodi	289.56 (523)	287.22 (490)	288.43 (1013)
2	Agricultural Institute, Anand	289.05 (38)	287.11 (37)	288.08 (76)
3	Palace Dairy Farm, Baroda	284.73 (99)	282.38 (87)	283.63 (186)
	Average/Total	288.81 (660)	286.53 (614)	287.71 (1274)

As in all the other breeds studied, the bull calves were carried on an average, for a longer period than heifer calves. Charodi Kankrej cows had the highest mean gestation period (288.43 days) and the Baroda ones the least (283.63 days). In the detailed analysis of variance, the Anand figures could not be included since they were scanty. Results of the analysis is summarised in table XI.

TABLE XI

Analysis of variance (Kankrej)

Source of variation	D. F.	M. S.
Between farm	1	173.65**
Between gestations	6	19.29
Interaction		
Farms × gestations	6	20.68
Between sexes	1	50.28*
Interaction		
Sexes × Farms	1	1.02
Sexes × gestations	6	8.50
Sexes × Farms × Gestations (Error)	6	6.58

* Significant at 5% level

** Significant at 1% level

Once again the variance in mean gestation period of *Kankrej* due to farms was statistically significant. So also was the variation due to sex of the calf carried. Parity and the interactions studied exerted no marked influence on the behaviour of the mean gestation lengths.

Discussion

In all the five breeds studied by us, a significant variation in gestation period was observed in herds of a given breed raised at different farms. This variation without exception was also statistically significant. It is known that reproductive disorders when present in herds, affect their gestation lengths (White, Rettger & Chapman, 1923 ; White, Rettger & Macalphine, 1924 and Williams, 1943). This is particularly so of herds with known histories of abortion, retention of afterbirth, dystocia and other interferences with normal pregnancies. In the herds considered by us generally, there were no such serious histories and as such the influence of infection factor on gestation periods could be ruled out. Differences in climate and geography are known to produce a marked effect on gestation period through changes in the normal seasons of calving. Thus Ineichen (1946) reported a seasonal influence in the gestation periods of *Brown Swiss* cattle. Similar influence of season of calving on gestation lengths was noticed by Hermann and Spalding (1947) and Brakel, Rife and Salisbury (1952). While the season of calving undoubtedly could have produced some effect in herds like *Sindhi* in our data since this breed had been raised under diverse conditions, in other cases notably *Kankrej* and *Tharparker* (the Sakrand figures were omitted from the analysis), such an influence might be taken as small. Yet, the existence of a distinct herd to herd variation in gestation period in all the breeds suggests that the possible cause has to be sought elsewhere.

Perhaps in the genetic constitution of the herds on the farms, the possible cause for wide variation in gestation period ultimately lies. During (1937) had observed a significant difference in the gestation period among the progeny of 7 bulls of *Swedish Fresian* cattle. Willett (1951) noted a strong positive correlation between the gestation period of calves sired by a particular bull and that of the bull itself. Brakel, Rife and Salisbury (1952) had observed significant inter-sire difference in gestation period in the three breeds studied by them. They also noted a significant correlation between the time the dam and the time her individual progeny spent *in utero* in 100 *Ayrshire* and 100 *Jersey* gestations. Anant Krishnan, Lazarus and Rangaswamy (1952) had noted in *Sindhi, Gir, Tharparker, Hariana, Sahiwal* and some crossbred cows, the significant influence which the sire of the calf exerted on gestation period. Thus while *Ayrshire* cows with *Ayrshire* calves *in utero* showed an average gestation period of 277.70 days and *Sindhi* cows with *Sindhi* calves *in utero* —a gestation period of

283.22 days, the same *Sindhi* cows carried their *Ayrshire* × *Sindhi* calves for 277.03 days. Most of the herds included in our study were originally built up from small importations from a common source or region and subsequently increased through local purchases. Due to the recent development of pedigree breeding and total absence of closed herds in our country, this common source will not obviously be genetically pure. Close breeding from samples of animals taken from a source of this type is expected to give varying results depending upon the particular sets of genes affecting gestation period received by them and the later segregation of these genes. Even where the common source could be taken as comparatively pure as for example in European breeds of cattle, Veiga, Paiva and Chieffi (1947) had found in case of *Freisians* reared in Brazil a significant difference in gestation period from those kept elsewhere; Ward and Castle (1948) noted a marked difference in duration in pregnancy for animals raised at Waikato district as compared to those raised in two other districts studied by them. Further the use of sires of unknown pedigree as for example those purchased at cattle fairs in India and chance matings with bulls of other breeds sometimes kept on the same farm could have jointly or severally contributed to the observed variation in gestation periods.

Coming to the question of influence of parity *i. e.* the chronological order of gestation on pregnancy duration, with the single exception of *Hariana*, in none of the other breeds investigated any noticeable effect could be detected. In all probability the significant difference noticed in *Hariana* might be due to the existence of two distinct strains in the breed. Originally developed as a heavy draught breed, milk genes had been introduced into this breed subsequently, so that we have at present two distinct types, one purely draught animals and the other combining draught and milk qualities. Depending upon the particular sets of genes received at segregation, a difference in gestation lengths depending upon the particular type of bulls used in different gestations, may be reasonably expected. Lazarus and Anantkrishnan (1952) who worked on the Bangalore data (included in our study) obtained results very similar to ours. Among foreign breeds, Blum (1939), Dinkhauser, Trampen and Bergmann (1944), Hermann and Spalding (1947) and Brakel, Rife and Salisbury (1952) reported longer duration of pregnancy in older than younger cows, while Weaver, Wettstein and Harwood (1947) found that the first gestation was no shorter than the subsequent ones. Similar results as obtained by Weaver *et al. (loc. cit.)* and by us for *Sahiwal, Tharparker, Sindhi* and *Kankrej* were

found by Rageb and Asker (1951) for Egyptian cattle, buffaloes, *Short horn* and *Short horn* grades.

A uniformly longer duration of pregnancy for male as opposed to female carrying gestations was seen by us in all the breeds studied. The difference was statistically significant in all cases. Among foreign breeds Long, Girlaugh and Rife (1948), Ward and Castle (1948), Jaffar, Chapman and Casida (1050), Burries and Blumn (1952) and Brakel, Rife and Salisbury (1952) to mention a few among recent workers, found that in the breeds studied by them, in the majority of the cases the male calves were carried for a longer period than female ones. This is also our finding for Indian cattle. As opposed to this, Livesay and Bee (1945) and Grossman and De Oliveira (1949) reported that the sex of the calf had no appreciable influence on the gestation length. Further, Burries and Blumn (1952) reported in *Short horns* and Wheat and Riggs (1952) in Aberdeen-Angus that the *female* calves were carried for a slightly longer period than bull calves. These results indicate the existence of breed to breed difference in the influence of sex of the calf on gestation period.

Summary

1. The influence of geographical location, sex of the calf and the chronological order of gestation on gestation period of five Indian cattle breeds namely, *Sahiwal*, *Tharparker*, *Sindhi*, *Hariana*, and *Kankrej* was studied from records collected from different farms in India and from territories now in Pakistan.

2. A significant difference in duration of pregnancy among farms within breeds was noticed. This has been described as possibly due to the genetic impurity of original source from which the animals had been imported for building up herds and the possible use of bulls of unknown pedigrees purchased at fairs or shows.

3. With the exception of *Hariana* in none of the other breeds any evidence of significant influence of chronological order of gestation on gestation period could be detected. The marked variation observed in *Hariana* is suggested as possibly due to the existence of two distinct strains in this breed.

4. In all breeds, the male calves were carried for a longer period than the female calves. The difference was statistically significant,

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EFFECT OF MONTH OF CALVING ON THE GESTATION PERIOD OF INDIAN CATTLE

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IN an earlier paper (Prabhu *et al*, 1961) the effect of geographical location, sex of the calf and parity on the gestation of *Sahiwal, Tharparkr, Hariana* and *Kankrej* was considered. It was observed that in all the breeds studied, the bull calves were carried for an appreciably longer period than heifer calves; that with the exception of *Hariana*, the chronological order of gestation (parity) had no effect on duration of pregnancy, and that the gestation period within a breed varied significantly with farms. The last mentioned fact was suggested to be probably due to the genetic impurity of the original source from which the foundation cows had been imported and the probable subsequent use of purchased bulls of unknown pedigree. In the present paper, the effect of month of calving on gestation period is considered.

Material and Method

Full particulars of the data on which the present study is based was given in the earlier paper (Prabhu *et al*., 1961). Due to lack of sufficient information when the data was arranged according to the month of calving an analysis according to breed was not attempted to begin with. All available information on the gestation periods of Indian cattle irrespective of breed was pooled together and then arranged according to the month of calving according to order of lactation and sex of the calf born, and an overall analysis of variance carried out utilising the mean gestation period values.

Results

The basic data is presented in Table I and the summary of the analysis of variance in Table II. With one exception, the mean values used in the analysis were calculated from a minimum of 17 gestations. The analysis showed that a significant variation existed between months of calving *i. e.* the gestation periods had varied significantly according to the month in which the calves were dropped, or, indirectly according to the months in which the conceptions took place.

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TABLE I

Mean gestation period (in days)

( ) Number of observations

Number of gestations	MONTHS OF CALVING							
	January		February		March		April	
	♂	♀	♂	♀	♂	♀	♂	♀
1	286.79 (170)	286.13 (202)	287.21 (134)	285.50 (177)	287.70 (168)	285.36 (159)	287.14 (152)	285.99 (119)
2	287.60 (127)	286.05 (123)	286.98 (122)	285.75 (124)	288.55 (95)	285.30 (103)	288.03 (100)	285.36 (102)
3	288.04 (101)	285.39 (98)	288.96 (71)	286.52 (89)	287.95 (88)	286.86 (77)	290.36 (73)	286.10 (78)
4	287.15 (60)	286.18 (74)	287.54 (59)	286.98 (53)	287.25 (51)	286.31 (42)	286.50 (48)	284.06 (54)
5	285.74 (35)	287.22 (51)	288.00 (40)	287.37 (49)	286.59 (39)	286.00 (23)	287.11 (27)	286.44 (27)
6	287.73 (26)	285.94 (34)	288.05 (37)	285.11 (28)	285.16 (25)	285.71 (34)	288.09 (22)	285.46 (24)

TABLE I (Contd.)

( ) Number of observations

Number of gestations	MONTHS OF CALVING							
	May		June		July		August	
	♂	♀	♂	♀	♂	♀	♂	♀
1	285.78 (129)	283.24 (144)	285.17 (75)	284.11 (74)	287.05 (95)	286.04 (102)	287.45 (89)	285.76 (91)
2	287.30 (93)	284.42 (79)	286.58 (64)	285.62 (58)	288.76 (74)	285.78 (78)	287.33 (97)	285.17 (75)
3	287.84 (56)	284.43 (72)	285.95 (56)	285.08 (60)	287.45 (51)	285.61 (67)	285.62 (65)	286.88 (81)
4	285.97 (33)	285.42 (33)	287.59 (51)	286.73 (44)	287.90 (39)	286.53 (34)	285.62 (47)	286.88 (43)
5	286.94 (34)	286.30 (23)	287.16 (38)	285.17 (24)	286.92 (36)	285.48 (33)	286.68 (31)	285.97 (36)
6	287.82 (17)	284.72 (18)	286.89 (18)	288.14 (21)	287.82 (22)	286.55 (22)	288.42 (24)	283.65 (23)

TABLE I (Contd.)

( ) Number of observations

Number of gestations	MONTHS OF CALVING							
	September		October		November		December	
	♂	♀	♂	♀	♂	♀	♂	♀
1	285.96 (84)	287.12 (64)	286.20 (83)	285.48 (84)	280.32 (109)	284.26 (91)	279.33 (132)	285.94 (133)
2	286.84 (88)	284.40 (87)	287.19 (78)	284.50 (80)	280.50 (88)	282.79 (101)	287.09 (157)	285.17 (108)
3	286.86 (66)	283.14 (65)	286.57 (63)	285.61 (89)	289.67 (90)	285.13 (70)	286.32 (106)	284.86 (100)
4	285.71 (55)	284.64 (44)	287.06 (49)	284.28 (40)	286.68 (94)	285.77 (57)	285.58 (62)	283.44 (75)
5	284.54 (24)	283.75 (28)	284.73 (41)	285.06 (35)	287.97 (32)	284.50 (52)	286.35 (31)	284.61 (44)
6	285.36 (28)	283.66 (25)	285.62 (21)	284.33 (30)	287.78 (23)	285.11 (28)	283.03 (14)	285.81 (31)

TABLE II

Analysis of Variance

Source of variation	D. F.	S. S.	M. S.	F. Ratio
Between months	11	64.16	5.83	5.34**
Between gestations	5	10.55	2.13	1.95
Months × gestations	55	48.37	0.88	
Between sexes	1	76.84	76.84	7.05*
Sexes × months	11	22.54	2.05	1.88
Sexes × gestations	5	11.14	2.23	2.04
Sexes × months × gestations (Error)	55	60.46	1.09	
Total	143	294.16		

* Significant at 5 per cent level.
** Significant at 1 per cent level.

Discussion

Conflicting findings are reported in literature on the effect of month of calving on the duration of pregnancy in cattle. Thus in *Bos taurus*, Jakubec (1941) working on material from 13 breeds from 4 separate territories found no correlation between average duration of pregnancy and season of calving. Dinkhauser *et al.* (1944) who studied 203♂♂ and 244♀♀ calves born in the Friedland herds since 1922 found no essential differences in length of gestation period of calves born during July–October and those born during January–April. The authors ascribe this as due probably to the uniform level of feeding maintained at Friedland. Ward and Castle (1948) found that the month of conception had no influence on gestation period of New Zealand dairy cattle. In like manner, no effect of season of calving on length of gestation was noticed by Jafar *et al.* (1950) in American Dairy cattle. As opposed to this Ineichen (1946) observed that in *Brown-Swiss* cattle pregnancy was shorter in cows that conceived in February and longer in cows that conceived in August. Herman and Spalding (1947) found in American dairy cattle the gestation period was 1-3 days longer for cows calving in autumn and winter than for cows calving in Spring and summer. Ragab and Asker (1951) noted a significant effect of month of calving on length of gestation in Egyptian cattle. Lambards (1951) who studied 25,356 live births of Black Pied Lowland cows found that births during June-November occurred after a mean gestation period of 278.33 *v* 280.16 for births during December–May.

In Indian cattle, the findings of Lazarus and Anantakrishnan (1952) showed no significant influence of the month of freshening on the gestation period of *Sindhi* and *Gir* cows. Their conclusions were based on the study of 1,093 *Sindhi* and 359 *Gir* gestations. The author's findings based on 9,645 gestations of Indian cattle breeds, on the contrary, showed *significant* variation in the duration of gestation period depending upon the month of freshening. Cows calving in February had the longest gestation period (287.00 days) and those freshening in September the shortest (284.74 days). The difference was statistically significant. In general, it was found that shorter gestations were found in cows freshening during September, October, November and December months and longer gestations in those calving during the remaining months. Since the former get plenty of grazing during the period of greatest foetal development in the second half of pregnancy, and the latter less or no grazing during like period, the differ-

ence in the mean duration of gestations in the two cases may be due to this factor. Ineichen (1946) in *Brown Swiss* cattle found a similar situation. Before drawing final conclusions, it is desirable to see whether this is so in each breed, since our calculations were based on pooled data over breeds.

Summary

1. Using means of gestation periods of Indian cows pooled over farms and breeds, an analysis was conducted to find out the effect of month of calving on the duration of pregnancy.

2. A total of 9,645 gestations were considered.

3. Results showed that a distinct month to month variation in duration of pregnancy exists, the cows freshening in the months of September, October, November and December having shorter gestations than those calving in other months.

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ON THE LIPASE ACTIVITY IN THE DEVELOPING CHICK BRAIN

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IT is now well established that in ontogenesis, the differentiation of an enzyme in an organ is directly related to the differentiation of function (Moog, 1952; George and Iype, 1959; 1960). In the chick embryo, the lipase activity of the heart and the rate of heart beat have been shown to be directly related (George and Iype, 1959). In the liver too, lipase activity varies in accordance with the functional changes in the organ, the highest being attained at the time when the embryo takes over to fat as the energy source for its development (George and Iype, 1960). Unlike the heart and liver which are actively functional during development, the brain is called upon to function at, or after hatching (Moog, 1947).

The main energy source for the activity of the adult brain is carbohydrate, chiefly glucose (Kety, 1957; McIlwain, 1959). However, cerebral tissues can oxidize several fatty acids, and lipids may be oxidized by the brain and probably used as a source of energy (Geyer *et al*, 1949; Weinhouse *et al*, 1950; Geiger *et al*, 1956; Vignais *et al*, 1958). During development the chick embryo is also known to pass through an ontogenic sequence of food stuffs for energy, carbohydrate, protein and fat (Needham, 1931). Since the adult brain could utilize fat and so also the whole embryo in later stages of development, it is logical to assume that the developing brain also may utilize fat at least to a limited extent. The present study was therefore undertaken to look for the presence of a lipolytic enzyme, lipase, and to assess quantitatively the extent of its activity in the chick brain during development. Certain properties of the enzyme were also studied using some of the known activators and inhibitors of the pancreatic lipase, a lipase which has been extensively studied by earlier workers.

Material and Methods

The tissue was taken from the same embryos which were used for our study on the liver (George and Iype, 1960). White Leghorn eggs of uniform size were incubated at 100 ± 0.02°F. After the desired period of incubation, the embryos were carefully separated from the yolk. Brain of

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the embryos from the 8th day upto the 19th day of incubation was used for the quantitative determination of lipase activity. Upto the 12th day of incubation, the tissue from more than one embryo was pooled for each experiment. The brain was dissected out with a pair of fine watchmakers' forceps and the traces of blood were removed by blotting gently with filter paper. The whole brain including the medulla oblongata was used. After weighing quickly, the tissue was homogenized in distilled water in cold, and again diluted with cold distilled water so that each ml. of the homogenate contained 40 to 50 mg. wet weight of the tissue. The lipase activity of the homogenate was determined manometrically at pH 7.4 using the Warburg apparatus (adapted from Martin and Peers, 1953). An emulsion of tributrin (4% v/v) in 0.0148 M $NaHCO_3$, prepared by shaking in a conical flask with a drop of "Tween 80" was used as substrate. The reaction flack contained 1.5 ml. of 0.025 M $NaHCO_3$ and 1 ml. of homogenate in the main chamber and 0.5 ml. of substrate in the side arm, making a total volume of 3 ml. This gave a final concentration of 0.0148 M $NaHCO_3$. In a gas phase of 5% CO_2, this concentration of $NaHCO_3$ gave a pH of 7.4 (Umbreit *et al*, 1957). The flasks and manometers were gassed with a mixture of 5% CO_2 in Nitrogen for 3 minutes and equilibrated for 10 minutes in the water bath at 37°C. The readings were taken at regular intervals for one hour, after tipping in the substrate and equilibrating for another 3 minutes. The manometers were shaken at 115–120 oscillations per minute with an amplitude of 5 cms. The lipase activity in the brain is expressed as the number of μl CO_2 / mg. protein / hour. Protein was estimated according to the micro-Kjeldahl steam distillation method (Hawk *et al*, 1954).

For studying the effect of activators and inhibitors, the enzyme homogenate was prepared as described earlier, and then centrifuged for 5 minutes at about 1500 $\times g$. The supernatant was used as the enzyme solution. The enzyme activity was determined as described above. The reaction flasks contained 1.5 ml. of 0.025 M sodium bicarbonate, 0.5 ml. of the substance under test in concentrations to give the final concentrations tried and 0.5 ml. of the enzyme material in the main chamber and 0.5 ml. of substrate (4% v / v tributryin in 0.0148 M sodium bicarbonate emulsified by shaking with a drop of "Tween 80") in the side arm. The test solution was introduced into the reaction flask before the enzyme was added. The rest of the procedure was just the same as outlined earlier. A control was run for each experiment in which 0.5 ml. of distilled water was added

instead of the solution under test. Effects of sodium taurocholate, sodium fluoride, eserine sulphate, protamine sulphate and heparin were studied.

Results and Discussion

Fig. 1

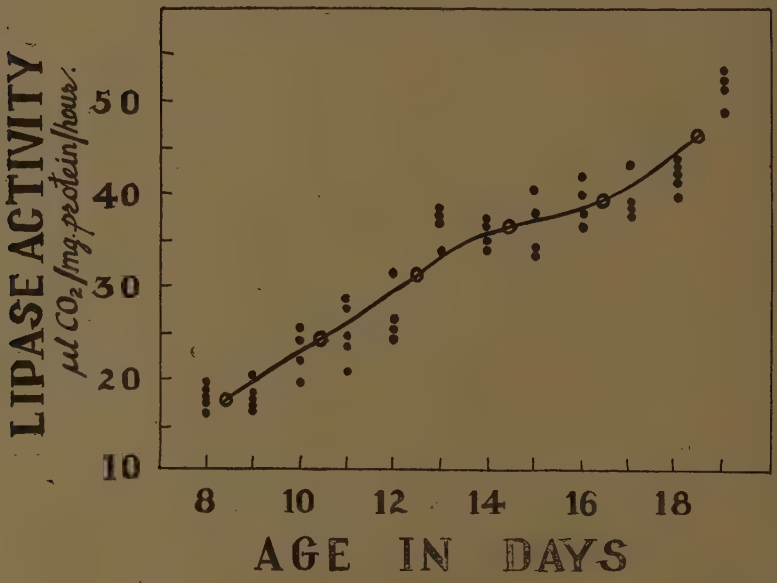

Fig. 1 Graph showing the quantitative changes in lipase activity in the chick brain during development. Dots indicate the individual readings, and circles represent the average readings given in table. 1.

TABLE I

LIPASE ACTIVITY IN THE CHICK BRAIN DURING DEVELOPMENT

Age in days	Lipase activity (μl. CO_2/mg. protein/hour)	Number of Experiments
8 – 9	17.85 ± 1.00	10
10 – 11	24.05 ± 3.05	9
12 – 13	31.57 ± 6.08	8
14 – 15	36.13 ± 2.48	8
16 – 17	39.47 ± 2.19	8
18 – 19	46.29 ± 5.31	9

The lipase activity of the brain increased gradually from the 8th to 19th day of incubation without much fluctuation (Fig. 1 ; Table 1). The

lipase activity in the chick heart and liver (George and Iype, 1959, 1960). during development showed considerable variations with respect to the different stages of development thereby indicating a relationship between these variations and the functional changes taking place in these organs. The brain lipase on the other hand followed a gradual rise in activity. This difference leads to the conclusion that unlike that in the heart and liver, in the brain the level of catabolic activity is considerably low and is therefore indicative of the much limited functional status of the organ.

The brain is known to contain a good amount cholinesterases. Mendal and Rudney (1943) found that the brain tissue (mouse, dog) which effectively hydrolyses acetylcholine, exhibits some activity towards tributyrin and tripropionin. It is generally believed that the enzyme hydrolysing tributyrin is a true lipase (Dunkley and Smith, 1951) even-though there is a little overlapping in its substrate specificity. In our study with some activators and inhibitors of pancreatic lipase the following results were obtained.

Sodium taurocholate (Fisher Scientific Co.) was found to inhibit the enzyme by about 80% at a concentration of 10^{-3} M. Sodium taurocholate is an activator for pancreatic lipase (Nachlas and Seligman, 1949), but according to Mattson and Beck (1956) bile salts have little effect on the hydrolysis of triglycerides. George and Scaria (1959) studied the properties of the pigeon pancreatic lipase and pigeon breast muscle lipase and found that sodium taurocholate activates pancreatic lipase but inhibits the muscle lipase. They also have shown that the intermediary metabolites and adenosine triphosphate inhibit the pancreatic lipase, but activates the muscle lipase, and suggested that these enzymes are adapted for maximal activity in their own respective physiological environment where they occur. Further evidence to this is obtained from our studies on the liver lipase of developing chick which have shown that this lipase is activated by bile. However, the liver lipase also is inhibited by 10^{-2} M sodium taurocholate. Similar results were obtained for the adipose tissue lipase (George and Eapen, 1960), insect muscle lipase (George and Bhakthan, unpublished) and the pigeon heart lipase and the sheep heart lipase (George and Iype, unpublished). From the above results it seems probable that these lipases have some properties of their own which are not shared by the pancreatic lipase.

Sodium fluoride was found to inhibit the brain lipase completely at a

concentration of 0.2 M. Sodium fluoride is a complete inhibitor for pancreatic lipase also (Hollet and Meng, 1956).

Eserine sulphate at a concentration of 10^{-5} M was found to have no effect on the enzyme. This substance is primarily noted for its inhibitory effect in low concentrations on cholinesterase (Richter and Croft, 1942; Mendel and Rudney, 1943). It is also known to affect adversely the esterase activity of human serum (Nachlas and Seligman, 1949). Mendel and Rudney showed that eserine sulphate in the same concentration as the one we have used (10^{-5} M), has no effect on the hydrolysis of tributyrin and suggested that the enzyme responsible for the hydrolysis of acetylcholine has practically no share in the hydrolysis of non-choline esters.

Heparin at a concentration of 10 μg./ml. was found to have no effect on the enzyme. Protamine sulphate (0.05%) inhibited the enzyme by about 10%. Protamine sulphate was shown to inhibit the clearing activity of post heparin plasma by 60%, but the pancreatic lipase was not inhibited (Hollet and Meng, 1956). Heparin on the other hand activates lipoprotein lipase.

The action of sodium fluoride and eserine sulphate shows that the enzyme is like the pancreatic lipase and not of the nature of the cholinesterase or an unspecific esterase. The action of protamine sulphate indicates the presence of traces of lipoprotein lipase. On the contrary heparin which activates lipoprotein lipase has no effect on the brain enzyme. These conflicting results may be because of the fact that the concentrations of the substances that were tried might not act on the brain lipase as it acts on the pancreatic lipase. It might be that one enzyme is more sensitive than the other to some of the substances used. Moreover the enzyme material used was not a purified form.

Considering the action of all the substances used it could be concluded that the tributyrin hydrolysing enzyme of the brain is not an unspecific esterase or cholinesterase, but a lipase comparable to the pancreatic lipase, though having some properties of its own.

Summary

1. The lipase activity of the brain during development of the chick from 8 to 19 days has been determined manometrically.

2. The lipase activity was found to increase slowly all throughout the period studied without any striking fluctions.

3. The changes in the lipase activity of the brain during development is compared with that of the developing chick heart and liver.

4. The effect of some activators and inhibitors were studied and it is concluded that the enzyme is not an unspecific esterase but a lipase.

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A COMPARATIVE STUDY OF THE LIPASE ACTIVITY IN THE BLOOD SERA OF THREE REPRESENTATIVE BIRDS

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THE recent studies conducted in our laboratories on the structure and metabolism of the breast muscle of birds, have shown that in birds indulging sustained flight, fat is the chief energy fuel and that this muscle in such birds is structurally and functionally adapted for a high rate of aerobic metabolism of fat. It has also been shown that in the pectoralis of these birds there is a high concentration of a lipase (George and Scaria, 1956). Even in the same muscle where exist fibres of two types, one loaded with glycogen adapted for a glycolytic metabolism and the other loaded with fat adapted for an oxidative metabolism of fat, as are found in the pigeon breast muscle, it has been demonstrated that there is a high concentration of lipase in the fat loaded fibre while the enzyme could not be detected in the glycogen loaded one (George and Scaria, 1958; George and Iype, 1960). These studies have been recently reviewed by Drummond and Black (1960).

Similarly the heart which is an organ capable of continuous activity, is also known to use fat for energy (Bing, 1956). This organ has also been shown to possess a high lipase activity (George and Scaria, 1957; George and Iype, 1959). Bing *et al.* (1954) have shown that the myocardium extracts fatty acids from the coronary circulation.

In the light of the above studies we became convinced of the possibility of a lipase in the blood of these birds playing an important part in the mobilization of fat and the active transport of fatty acids to the muscles and other organs. It was therefore thought desirable to conduct the present study on the lipase activity in the sera of three representative birds, *e.g.* a non-flying bird, a good flier but non-migratory and a migratory bird. The domestic fowl (*Gallus domesticus*), the blue rock pigeon (*Columba livia*) and the rosy pastor (*Pastor roseus*), were chosen as the three representative types.

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The occurrence of a lypolytic enzyme in the blood of vertebrates is well known. Harriot (1896) noted the presence of an enzyme in the serum and tissues which hydrolzed monobutyrin. He called it lipase. Lypolytic activity of the serum with tributyrin has been noted by Alper (1953). In recent years, however, many authors have expressed doubt as to the occurrence of a true lipase in the blood. Korn (1955), Hahn (1943) and Engelberg (1958) described a heparin activated lipoprotein lipase. This lipoprotein lipase does not split tributyrin, nor does it enhance the tributyrin splitting capacity of rat and dog plasma. It could clear lipemic plasma in the almost complete absence of both an ali-esterase and a lipase. These findings show that the action of the clearing factor seems to be in every respect different from an ali-esterase or a lipase (Overbeek, 1955).

In our present study, we have estimated quantitatively the lypolytic activity in the sera of the above birds and we have regarded the enzyme as a lipase which is distinctly different from the lipoprotein lipase mentioned above.

Materials and Method

The fowls and pigeons used were domesticated laboratory birds. In the case of the former the hens used were in the period of egg laying. The rosy pastors were captured by trapping them by means of a mist net during the period of March to April and were sacrificed within 12 hours of captivity. These birds were in their premigratory period and would have started on their return journey within a few weeks' time.

In all the cases, blood was directly collected from the heart in clean centrifuge tubes and allowed to clot at room temperature for 10-15 minutes. The blood was not kept in the refrigerator because it would take longer time for clotting and moreover it was found that the enzyme activity was not reduced during the time when kept at room temperature.

The clotted blood was then centrifuged approximately at 2500 r.p.m. for 5–7 minutes and the clear supernatant serum was transferred to a chilled centrifuge tube. This serum was measured and diluted in cold distilled water and was used as the enzyme material in all the experiments. In the case of the rosy pastor 2–3 birds were sacrificed and the blood was pooled for each experiment.

The lypolytic activity was determined by a manometric method adapted from Martin and Peers (1953), using a bicarbonate carbon dioxide buffer system of pH 7.4 at 37°C in the Warburg apparatus (George, Vallya-

than and Scaria, 1958). The reaction flask contained 1.5 ml. 0.025 M bicarbonate buffer solution and 1 ml. of the enzyme in the main chamber and in the side arm 0.5 ml of tributyrin, emulsified by shaking 4% (v/v) tributyrin in 0.0148 M bicarbonate with a drop of 'Tween 80'. The flasks and the manometers were gassed with a mixture of 5% carbon dioxide and 95% nitrogen for three minutes. After 10 minutes equilibration in the Warburg bath the substrate was tipped and after another equilibration of three minutes the levels were adjusted and the readings were taken at regular intervals for one hour. The lipase activity is expressed as the number of μl CO_2 evolved / hour / ml. of serum.

Results

Table 1 giving the lipase values of the sera of the three birds studied

TABLE 1

Sex	Fowl	S. D.	Pigeon	S. D.	Rosy pastor	S. D.	Minimum No. of Expts.
Male	1189.87 ±	112.39	6824.25 ±	261.74	10699.00 ±	1207.81	4
Female	996.50 ±	65.78	5733.40 ±	849.32	12091.50 ±	504.4	4

Discussion

George and Jyoti (1955, 1957) showed that when the muscle of the pigeon is subjected to prolonged activity, fat is the chief energy source for the contraction of the muscle. George and Naik (1960) have estimated the intramuscular store of fat in the breast muscle of a number of different birds and have shown that those birds indulging in sustained flight have a much higher fat store in their breast muscle than the others. The lowest figure is of course for the domestic fowl which is a nonflying bird. George and Scaria (1958) showed the presence of a high concentration of a fat synthesizing and hydrolyzing enzyme, lipase in the breast muscles of flying birds. During prolonged flight as in migration, the fat store in the muscles cannot be expected to supply all the energy required and so additional supplies should come from the liver and the adipose tissue. That migratory birds store up fat in the body prior to migration (Odum and Perkinson, 1951; MaGreal and Farner, 1955), and that fat is reduced during migration (Williamson 1952, 1955) are well known. George and Jyoti (1953, 1955) have shown that in the pigeon, there is a reduction in

the liver fat also when the breast muscle is subjected to prolonged activity. In the light of these observations it is logical to expect a considerably higher fat content and lipase concentration in the blood of flying birds than in the nonflying ones. George and Menon (1954) reported the fat content of the fowl and pigeon bloods as 0.38 gm. and 1.76 gm. respectively for 100 ml. of whole blood. We have observed (unpublished) that in the rosy pastor too the blood has a high fat level. Also in the present study on the lipase activity in the bloods of three birds one sees the same trend in that the values obtained for the rosy pastor and the pigeon are very much higher than that for the fowl. The considerable higher lipase activity in the rosy pastor blood is interesting and significant in the light of the fact that the rosy pastor is a migratory bird.

However, if one looks at the figures obtained for the two sexes in the three birds studied, one sees a difference. This difference cannot be considered as a distinct sex difference which is statistically significant since the range of variation is rather wide as could be seen from the standard deviation.

Summary

Lipase activity in the blood sera of three representative birds e.g. a non-flying bird (domestic fowl), a good flier (pigeon) and a migratory bird (rosy pastor) has been studied. The highest concentration of the enzyme was obtained for the rosy pastor blood, next for that of the pigeon and the least for the fowl blood. The physiological significance of the variation in lipase activity in the bloods of the three birds studied, is discussed.

Acknowledgement

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INFLUENCE OF DIETARY CAROTENE LEVELS ON SEMEN PHOSPHATASES IN DAIRY BULLS

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THE physiological significance of phosphatases have attracted considerable interest during the last decade. Elevated Serum phosphatase is generally accepted as indicating bone disease or liver damage. Semen is a rich source of this enzyme (Reid *et al*, 1948). In bull semen the occurrence of the acid and alkaline phosphatase was reported by Reid *et al*, (1948); and Haq and Mullen (1949). Semen owes its powerfull phosphatase activity mainly to the seminal plasma which carries several dephosphorylating enzymes derived from the male accessory organs of reproduction. In addition, seminal plasma contains 5 nucleotidases, a pyrophosphatase and several adenosine triphosphatases as observed by Mann (1954). The level of the acid phosphatase is an important secondary sex characteristic and a certain correlation appears to exist in adult man between the level of phosphatase and androgenic activity (Gutman and Gutman, 1941). Alkaline phosphatase like the acid enzyme is widely distributed in the male accessory organs, (Mann, 1954). Bullsemen has low acid phosphatase activity but high alkaline phosphatase activity (Haq and Mullen, 1949). Alkaline phosphatase has an optimum pH of 9 and is capable of hydrolysing among others 1—phospho-fructose, 6—phospho fructose and 1 : 6 diphosphofructose : This indicates that this enzyme activity may represent an essential step in the process of fructose formation and secretion by accessory organs (Mann, 1954). Diet is reported to affect the phosphatase activity (Taylor, *et al.* 1952; Lawrie and Yudkin, 1949; Allison, 1956). Allison (1956) demonstrated a diminished alkaline phosphatase activity in all tissues and bonelesions which are indistinguishable from rickets. Ludwig (1954) showed in his investigation that alkaline phosphatase was reduced in the epiphyseal junction of the knee joints of young rats fed a vitamin A deficient diet. Feeding high vitamin A produced an increase in alkaline phosphatase in bones where epiphyseal junction had previously been ossified.

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Materials and Methods

In investigation reported here includes data from eight dairy bulls of which three pairs were identical twins and two unrelated bulls maintained by the Department of Dairy and Animal Husbandary, Oregon State College, U. S. A. These bulls belonged to a group of identical twin bulls, and were on vitamin A studies for about two years before this investigation was undertaken. Semen was collected once weekly according to the method described by Lambert and McKenzie (1940) and immediately diluted 1 : 50 with chilled physiological saline transferred to a water bath at 5°c and stored in the refrigerator. Enzyme determinations were carried out immediately as per the method described by Hawk *et al* (1951). A standard curve was ploted with various wave lengths and 630 millimicrons was chosen as best suited for measuring the enzyme activity. The results are expressed in Bodansky Units, one Bodansky Unit being two King and Armstrong Units.

Results

At least ten determinations were carried out from each experimental animal at an interval of one week. Mean alkaline and acid phosphatase activity in Bodansky Units for the entire period is given in Table 2. (vide figs. 1 & 2)

The data show that in the case of the control twin bulls no noticeable difference was observed between bulls in the enzyme activity of semen. The extremely high alkaline enzyme activity in the semen of control bulls is perhaps due to high energy intake in the form of molasses. The data from other bulls tends to show an appreciable increase in animal fed ' high carotene ' ration than that of ' low carotene ' intake.

The analysis indicates that bulls 266-B-1 and G-13-B-1 show a significant difference at 1% level in semen alkaline and acid phosphates activity between ' high ' and low carotene regime. A significant difference at 1% was also observed between semen of TW-1 and TW-2 in alkaline phosphatase levels. In case of bulls TV-1 and TV-2 a significant difference at 5% level was observed in the acid enzyme activity of semen. Considerable variation was observed between control and experimental bulls in their semen enzyme activity. Wide variation also exist between carotene fed bulls and between their ejaculates. The enzyme activity was practically similar among identical twins during pre-experimental period. This is further demonstrated by the close relationship between the control ident-

TABLE 1

Daily Carotene feeding schedule during experimental period.

Animal	Breed	Vitamin A intake	Pounds of Grain	Pounds of Hay	Remarks
1	2	3	4	5	6
BS-1	Brown Swiss	Normal feeding supplemented by Molasses			Identical twins
BS-2	,,	,,	,,	,,	,,
TW-1	Guernsey X Holstein	442320 I.U.	5	12	,,
TW-2	,,	42560 I.U.	5	12	,,
TV-1	Brown Swiss	484120 I U.	6	14	,,
TV-2	,,	45980 I.U.	6	14	,,
266-B-1	Jersey	353400 I.U.	5	7	Unrelated
G-12-B-1	Holstein	36632 I.U.	5	7	,,

TABLE 2

Mean alkaline and acid phosphatase levels in dairy bull semen in Bodansky Units/100 ml. semen.

Animal	Treatment	Alkaline phosphatase activity	Acid phosphatase Activity	Remarks
BS-1	Control	1248	270	Normal ration + Molasses
BS-2	,,	1127	261	,,
TW-1	'High Carotene'	477	206	
TW-2	'Low Carotene'	364	149	
TV-1	'High Carotene'	332	180	
TV-2	'Low Carotene'	265	137	
266-B-1	'High Carotene'	502	276	
G-13-B-1	'Low Carotene'	156	161	

TABLE 3

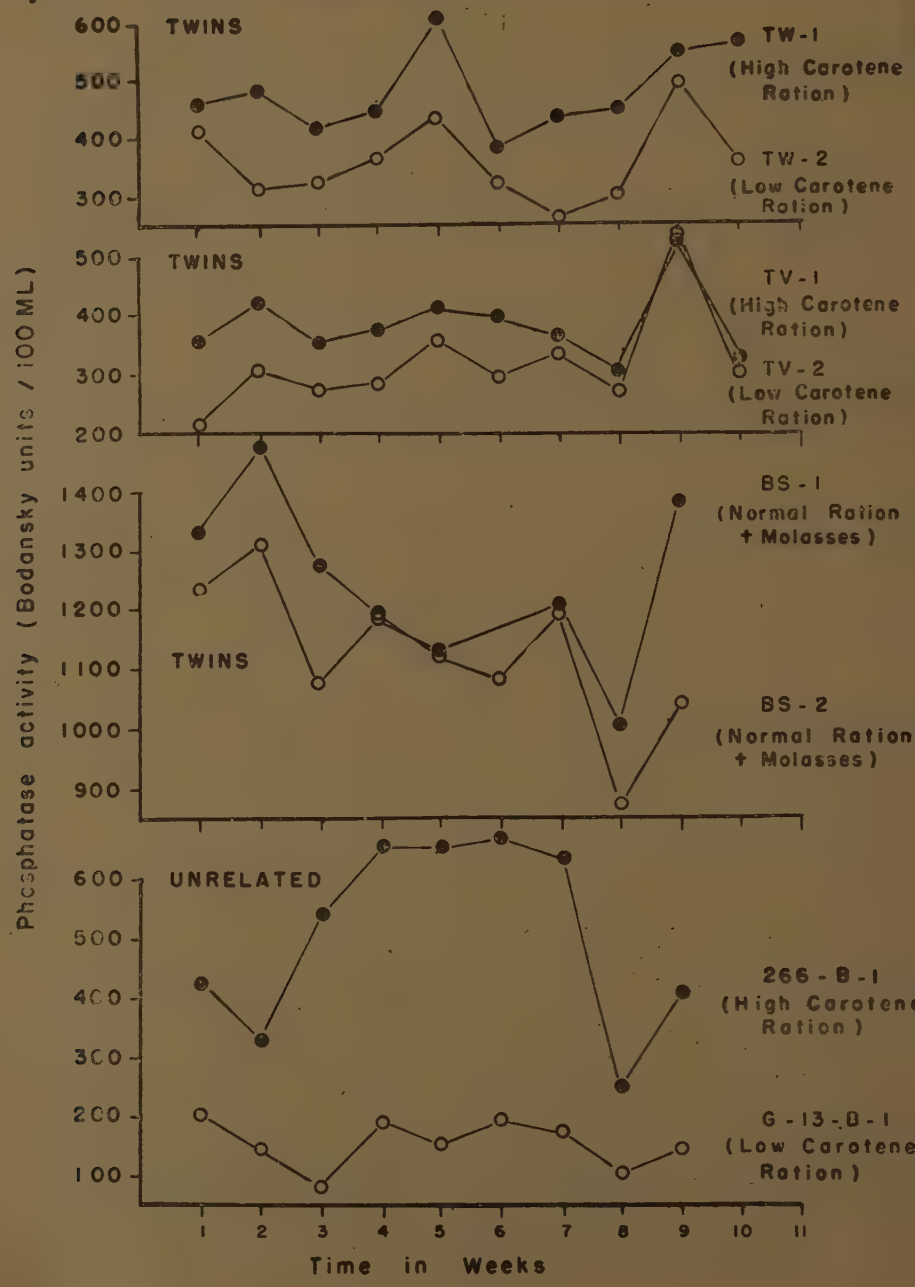
Value for acid and alkaline phosphatase levels in dairy bull Semen.

Animal	Treatment	*Alkaline phosphatase*		*Acid phosphatase*	
		t	df.	t	df.
BS-1-BS-2	Control	1.99	16	1.796	16
TW-1-TW-2	Treated	3.676**	16	1.535	16
TV-1-TV-2	,,	1.962	18	2.800*	16
266-B-1 G-13-1	,,	6.500**	16	3.088**	16

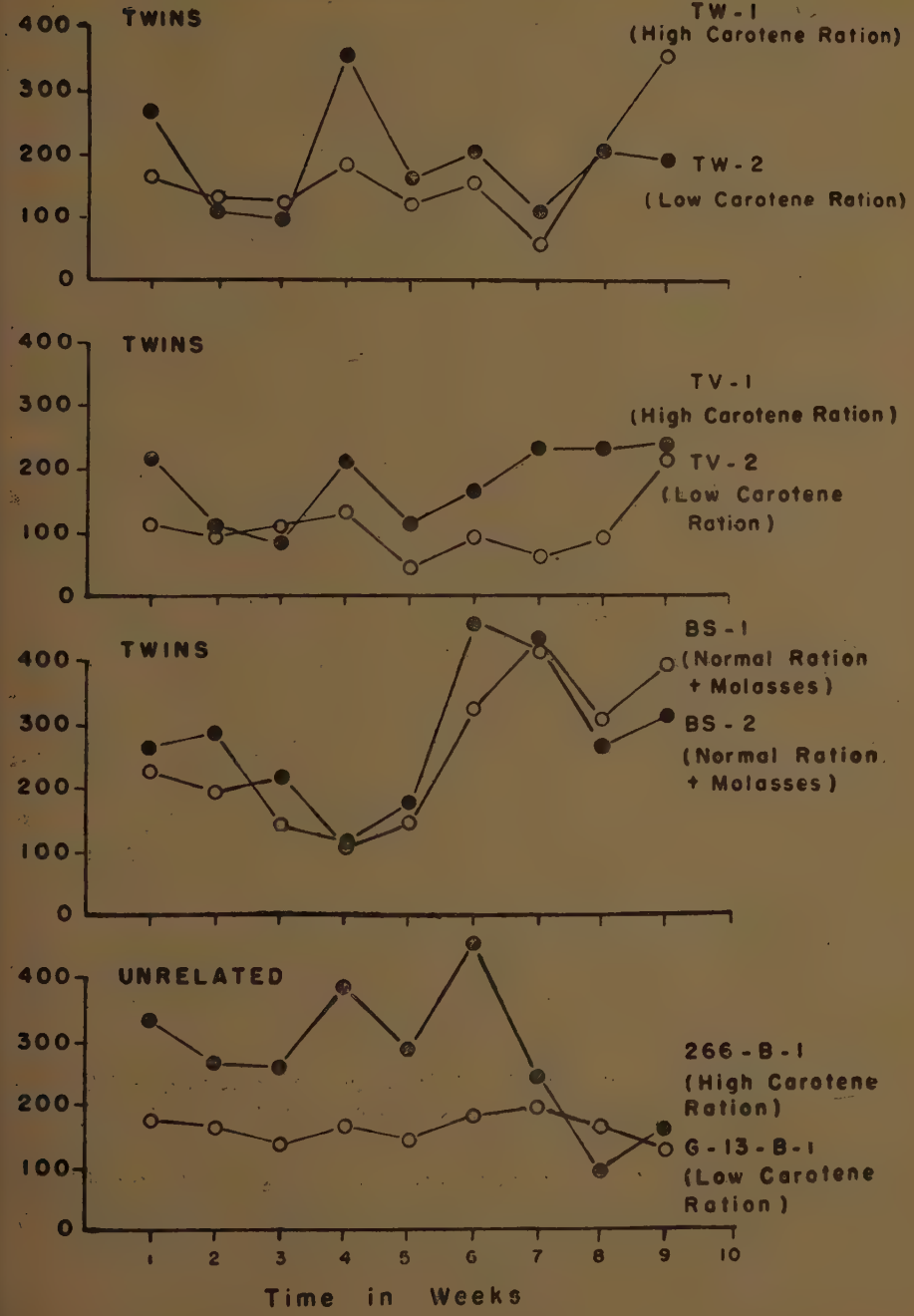
* Significant at 5% level

** Significant at 1% level.

Figure 1. Alkaline phosphatase activity in semen of dairy bulls



igure 2 Acid phosphatase activity in semen of dairy bulls



ical twin bulls in semen phosphatases levels. The alkaline phosphatase levels of high carotene fed bulls were not as high as control bulls indicating a significant effect of other dietary ingredients possibly molasses on Semen phosphatases.

Discussion

The findings in general agree with the study made by Reid *et al* (1948) who observed a striking dietary influence on alkaline and acid phosphatase in semen. The particular investigation consist of ten bulls which constitute two groups. Group I received a simple unsupplemented concentrate and Group II a complex concentrate supplemented with minerals and vitamins. Both groups received average grade of hay. The mean enzyme activity for Group I was 307.3 Units of alkaline phosphatase and 141.9 units of acid phosphatase (King and Armstrong Units/100 ml of semen) and Group II fed a complex concentrated mixture the alkaline enzyme activity was 477.6 and acid phosphatase of 198.8 units. This shows that the enzyme activity was higher in Group fed 'complex concentrate mixture' supplemented by minerals and vitamins. The findings certainly agree with the contention formulated in the study that diet certainly does influence the phosphatases in semen. The high level of alkaline phosphatase level of in control bulls could be attributed to feeding supplemented with molasses in ration. Further it is quite possible that dairy bulls would show significant differences in semen enzyme activity only in cases of extreme vitamin A deficiency in the diet. It is desirable to study this particular aspect in detail.

Summary and Conclusions

" High " carotene rations produced more alkaline and acid phosphatase activity in the semen of dairy bulls, than those of " Low " carotene intake.

A pair of identical twin bulls not on carotene feeding schedule, failed to show any significant difference in alkaline and acid phosphatase activity in the semen.

Acknowledgement

The study was completed at the Oregon State College, U.S.A. while the author was working with Prof. Fred. F. McKenzie, Department of Dairy and Animal Husbandry and was a part of the thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The author is grateful to Dr. F. F. McKenzie and Prof. Hugo Kruegar for guidance and suggestions.

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INFLUENCE OF DIETARY CAROTENE LEVELS ON PHOSPHATASE IN RABBIT SEMEN

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ALTHOUGH phosphatases are of paramount importance in nutrition they are not fully appreciated as entities in nutrition. They are undoubtly the more important of the enzymes, nutritionally, physiologically and pathologically. Acid and alkaline phosphatases belong to a larger group of enzymes known as phosphatases which catalyse the transformations of organic phosphatases (Doyle *et al* 1951). An observation that phosphatase activity in the human male urine is usually higher than that of the female, led Kutscher and Wolberg (1935) to examine the phosphatase in semen and in the prostate gland. They soon found that semen and prostate are among the richest sources of acid phosphatase in the human body. Investigations by Gutman and Gutman (1938) have shown that the level of the enzyme in the human prostate is low in childhood, but increases rapidly at puberty. The activity expressed in King and Armstrong units per gram of prostate tissue was 1½ units at 4 years of age, 73 units at puberty and 522-2284 units in adult men. A similar relationship to age was observed in monkeys and dogs. In both species administration of androgenic hormone to immature males stimulate considerably the output of the enzyme from the prostate gland (Gutman and Gutman 1939). Another important addition to our knowledge of the physiological function of acid phosphatase in the seminal plasma has been the discovery made by Lundquist (1946) that freshly ejaculated human semen contains phosphoryl choline which on ejaculation is rapidly dephosphorylated by acid phosphatase to free choline and orthophosphate. Of considerable interest also is the finding that acid phosphatase exhibits in *vitro* a distinct transferase activity (Green and Meyerhof, 1952).

Alkaline phosphatase like the acid enzyme is widely distributed in male acessory organs (Mann, 1954). Human semen with its conspicuously high level of acid phosphatase has a low concentration of alkaline phos-

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phatase. Bull semen on the other hand has only slight acid phosphatase but contains more of the alkaline enzyme. (Haq and Mullen, 1949). This difference between human and bovine semen is not altogether unexpected, since the bulk of bull seminal plasma is derived not from the prostate but from the seminal vesicle. Schivachman and Gould (1942) reported lowering alkaline phosphatase in Ginuea pigs on scorbutogenic diet. Rosenthal *et al* (1958) observed in protein depleted rats fasted four days that the liver arginase increased on an average 58% and liver alkaline phosphatase descreased 58%. Miller (1948) in his investigation with fasted rats demonstrated a loss of rat liver catalase, alkaline phosphatase and xanthine dehydrogenase. Haq and Mullen (1949) in their investigation on fertile, infertlie and bulls with testicular hypoplasia, observed that phosphatase activity goes down with testicular disease.

Materials and Methods

The investigation reported here, is based on the data from 18 New Zealand rabbits maintained by the Dairy and Animal Husbandary Department, Oregon State College, U.S.A. The eighteen New Zealand rabbits were assigned at random to three groups and semen collected twice weekly according to the method described by Macirone and Walton (1938). The experiment was conducted for about six months of which first three months constitute a pre-experimental period and other three months on experiment. During pre-experimental period the rabbits were fed pelletted feed and during three months on experiment they were fed a specially designed carotene free basal ration supplemented with required carotene levels. The composition of the basal ration is given in table 1.

TABLE 1

Composition of basal ration fed to rabbits during experiments period

Ingredients	Percent of Mixture	Remarks
Rolled Barley	25	Vitamin D 100 U.S.P. units/pound of feed
Ground oats	40	
Wheat Mill Run	10	Vitamin C. 100 I.U./pound of feed
Linseed Meal	22	
Ground Lime stone	2	
Iodized salt	1	

Vitamin D was provided by irradiated yeast which supplies 9,000 U. S. P. Units per pound of feed. Vitamin E in the ration was supplied by " Myvamix " yielding 20,000 I. U./pound of feed. The above feed constituents were thoroughly mixed and ground in feed mixer and grinder. To the ground feed was added carotene to give the resultant mixture carotone levels of 0, 10, 20 mg. per pound of feed. This served as a stock ration for the entire experimental period. The animals were tatooed in the left ear for easy identification and housed in separate cages. They were fed and given water daily and accurate records of daily feed consumption were kept for individual rabbits. The animals were closely watched for possible lesions due to Vitamin A deficiency. A weekly weight record was maintained throughout the experimental feeding period. Sexual response was noted individually by recording the time taken for first ejaculation.

TABLE 2

The mean phosphatase activity of rabbit semen pre-experimental feeding and during carotene feeding period in Bodansky units/100 ml. semen

	Alkaline phosphatase activity		Acid phosphatase activity	
Treatment	Prior to experimental period	During Experimental feeding period	Prior to experimental feeding period	During experimental feeding
Devoid of carotene	530	557	14.9	7.8
Low carotene	489	442	12.8	4.4
High carotene	503	492	12.7	5.2

From the above data in table 2 it is seen that in alkaline phosphatase, no noticeabie difference is observed between pre-experimental and experiment period. But in the case of acid phosphatase an appreciable difference is noticed in rabbit semen between pre-experimental and expeimental periods.

TABLE 3

The alkaline phosphatase activity in rabbit semen before and during experimental period subjected to analysis variance and t test

Treatment	t	df	Analysis of variance Prior to experimental period	df	Analysis of variance During the carotene feeding	df
Carotene free	0.780	5		2 and	F	2 and
Low carotene	0.501	4	0.718	272	5.342 **	266
High carotene	0.385					

** Highly significant

The above analysis indicate no difference between groups during normal feeding but a difference as significant as 1% is noticeable while the animals were on experimental feeding. But in the case of the acid enzyme a very drastic reduction in the enzyme activity in all groups during the experimental period was noticed.

TABLE 4

Acid phosphatase activity of rabbit semen subjected to analysis of variance and t test for pre and during experimental feeding period

Treatment	t	df	Analysis of variance Prior to experimental feeding period	df	Analysis of variance During experimental feeding	df
Devoid of carotene	3.341 *	5	F	2 and	F	2 and
Low carotene	4.988 **	4	1.725	234	1.848	234
High carotene	3.488 *	5				

* Significant at 5%
** Significant at 1%

The analysis clearly shows a difference in enzyme activity between pre-experimental and during experimental feeding periods. But no significant difference is observed between groups in acid phosphatase of semen either before or during experimental feeding period. As observed earlier there is a drastic reduction in the acid enzyme activity in all three groups while on experiment is seen from table 2. This could be explained by the fact that there was a drastic reduction in food intake and consequently animals lost weight and hence reduction in semen enzyme activity which could mask any effect of carotene feeding. A close inspection of data revealed an interesting phenomenon. There seems to exist a close relationship between weight loss and reduction in alkaline enzyme activity.

TABLE 5

Relationship between weight loss and loss of alkaline enzyme activity in rabbit semen

Animal No.	Group	Percent loss of weight at the end of experiment	Mean percent loss of weight for the group	Percentage of decrease in enzyme activity during experiment	Mean percent decrease for the groups	Remarks
2	No carotene	11	22.3%	10	19.3	
4		22		16		
7		30		6		
11		26		41		
12		29		25		
28		16		18		
3	Low carotene	18	19.8%	45	21.8	
5		27		20		
9		18		40		
24		20		—		
18		15		4		
6	High carotene	17	21%	33	21.2	
10		29		24		
15		22		15		
16		21		—		
17		19		27		
23		18		28		

The above data shows that for a certain percentage of loss of body weight there appears to be a similar decrease in the alkaline enzyme activity of semen in rabbits. The decreased alkaline enzyme activity of semen is proportional to the loss of body weight. (vide figs. 1 and 2)

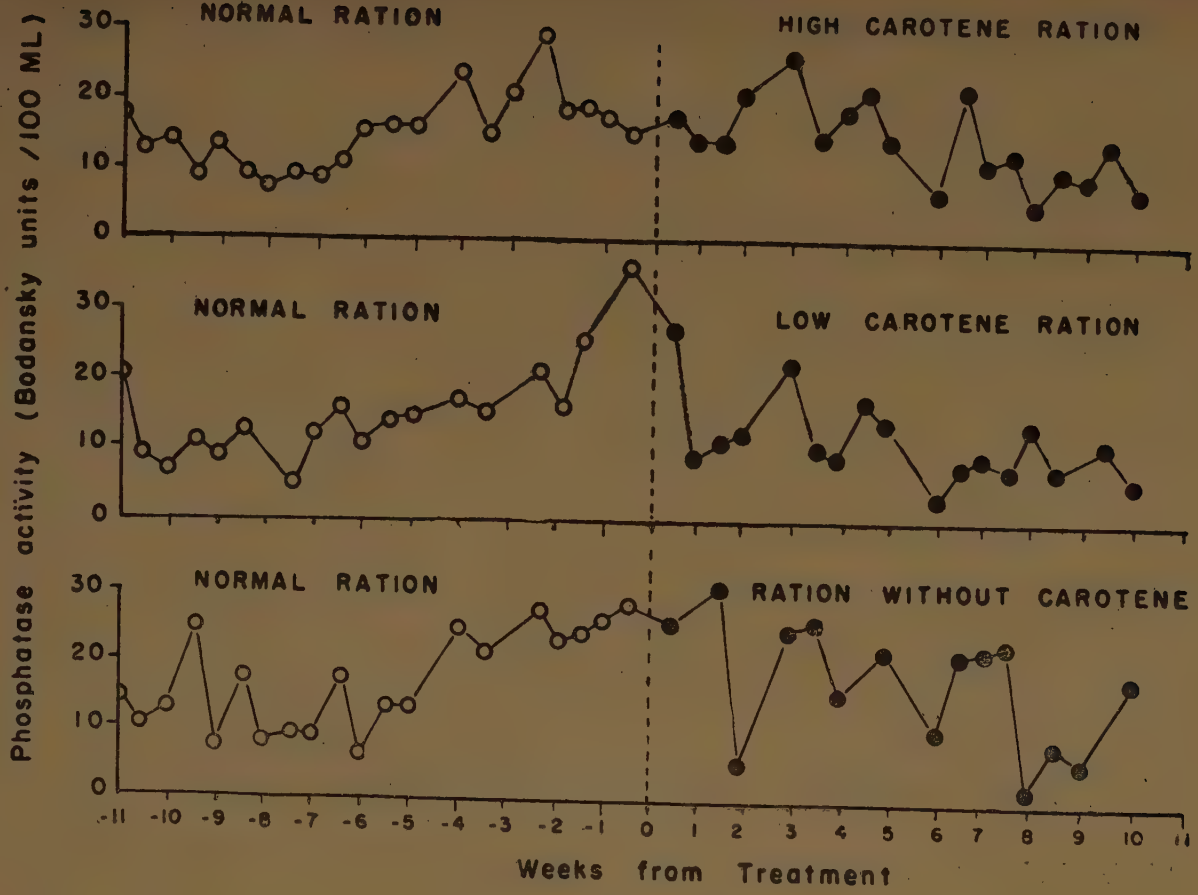
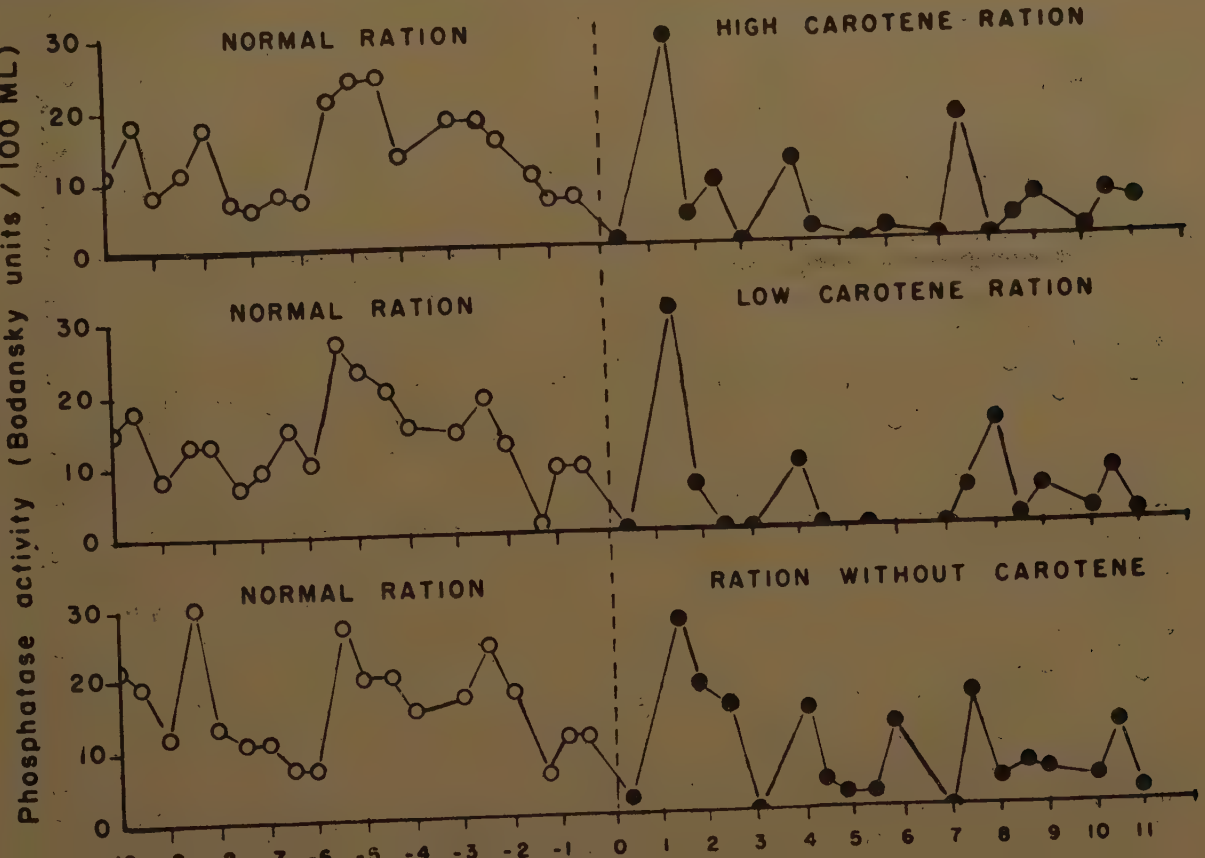

NORMAL RATION
HIGH CAROTENE RATION
NORMAL RATION
LOW CAROTENE RATION
NORMAL RATION
RATION WITHOUT CAROTENE
Phosphatase activity (Bodansky units /100 ML)
30
20
10
0
-11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
Weeks from Treatment

Figure 2. Acid phosphatase activity in rabbit semen before and after experimental ration



Discussion

The results obtained with rabbits do not agree entirely with the previous study made with dairy bulls. In the case of bull semen, appreciable difference is noticed between 'high' and low carotene fed bulls. But in the experiment with rabbit semen there was a general decrease in the alkaline enzyme activity irrespective of the levels of carotene fed to them. It is to be noted that there was a significant difference at 1% between groups during the experiment which clearly indicates a difference in response while on experimental feeding. But in the case of acid phosphatase activity in rabbit semen after treatment, agrees with the results obtained with dairy bulls although there was a decrease in the acid enzyme activity instead of an increase as observed in the previous investigation with dairy bulls. This decrease in acid phosphatase activity could be attributed to the weight loss. Alkaline phosphatase is a hydrolytic enzyme producing phosphoric acid and alcohol from the phosphate ester. Axelrod and Meyerhof have independently demonstrated that phosphatase may catalyse the transfer of phosphate residue from one organic molecule to another. The extremely high concentration of phosphatase in semen supports the fact that the enzyme has some role in sperm metabolism. The kidney, according to Danielli (1935), appears to be the regulating centre for phosphatase in blood. It is probable that kidney function might some how be hampered by Vitamin A deficiency which in turn affects the enzyme activity in the blood and subsequently affecting the phosphatase activity in semen.

Summary and Conclusions

The influence of dietary carotene levels on acid and alkaline phosphatase of eighteen New Zealand rabbits was considered.

There was a decrease in acid and alkaline phosphatase levels in rabbit semen when animals were fed three levels of carotene *viz.*, 0, 10 and 20 mg/pound of feed. It appears that for each percent loss of body weight, there was a similar percent decrease in alkaline enzyme activity.

Acknowledgement

The study was completed at Oregon State College, U. S. A. while the author was working with Professor F. F. Mckenzie, Department of Animal Husbandary and was part of the thesis submitted in partial fulfilment for the degree of Doctor of Philosophy. The author is grateful to Dr. F. F. Mckenzie and Professor Hugo Kruegar for guidance and suggestions.

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THE EFFECT OF STARVATION ON THE FAT AND PROTEIN CONTENTS AND THE LIPASE AND SUCCINIC DEHYDROGENASE ACTIVITIES IN THE RAT DIAPHRAGM

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IN our recent histophysiological study on the rat diaphragm (George and Susheela, 1961), we have described three distinct regions on the basis of certain structural and physiological features. These regions, the vertebral (dorsal) costal (lateral) and sternal (ventral) consist of two types of fibres: one white and broad, loaded with glycogen and the other red and narrow, loaded with fat. The relative distribution, diameter fat content, lipase and succinic dehydrogenase activities in these fibre types have also been studied and it was found that the fat-loaded fibres unlike the glycogen-loaded ones are better adapted for aerobic metabolism.

Since fat appears to be an important metabolite store in the rat diaphragm particularly in the red narrow fibres, it was thought desirable to study the changes during starvation in the fat and protein contents and the lipase and succinic dehydrogenase activities in the diaphragm.

Material and Methods

Wild rats (*Rattus norvegicus*) were freshly collected and used. Some were kept for 3 days deprived of food but not water. The others to be used as control were maintained on full diet. The rats were decapitated and complete diaphragms were immediately removed and spread on a clean filter paper. The three regions, vertebral, costal and sternal, each consisting of its right and left halves, were quickly cut out separately for quantitative estimations. For each estimation the same part from a number of individuals were pooled together since the material available from a few individuals was not sufficient.

Fat: For the estimation of the total fat content, the muscle was completely dehydrated by keeping in a hot air oven (80–100°C) for about 48 hours and then extracted with a mixture of 1 : 1 alcohol-ether using the

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Soxhlet apparatus. The percentage of fat content was calculated on the dry weight basis.

Protein: The protein content was estimated by the micro-Kjeldahl steam-distillation method (Hawk *et al*, 1954).

Lipase activity: The lipase activity was estimated quantitatively by the method of Martin and Peers (1953) as adopted in our laboratory (George, Vallyathan and Scaria, 1958) using the Warburg manometric apparatus with a bicarbonate carbon-dioxide buffer system of pH 7.4 at 37°C. The enzyme activity obtained is expressed in μl CO_2/evolved/mg. protein/hr.

Succinic dehydrogenase activity: The succinic dehydrogenase (SDH) activity was estimated by the colorimetric method of Kun and Abood (1949) using 2 : 3 : 5–Triphenyl–Tetrazolium chloride (TTC). The incubation medium consisted of 0.5 ml. of 0.1 M phosphate buffer of pH 7.4, 0.5 ml. of 0.2 M sodium succinate, 1 ml. of freshly prepared 0.1% TTC solution, and 1 ml. of tissue homogenate. After shaking well, the tubes were incubated for 2 hours in a constant temperature bath (37.°C). The enzyme activity was made to stop by adding 7 ml. of acetone. It was then centrifuged at 3000 r. p. m. The intensity of the colour of the clear supernatant was measured on a Klett-Summerson photoelectric colorimeter using a 420 mμ filter. The enzyme activity is expressed as μg formazan formed/mg. dry weight/2 hours.

Discussion

Changes in the metabolism during complete starvation have been studied by several investigators and it is known that starvation brings about a lowering of the heart rate, respiratory rate and muscular activity. Fat and protein (85% and 15% respectively) have been shown to be the two major energy sources during starvation in mammals (Terroine and Synephias, 1937). It has also been shown that during fasting 20–27% of the calories in case of the thigh muscle of rats of different ages were derived from protein and the rest from fat (Hagan and Scow, 1957). From the results obtained in the present study, it is evident that during starvation there was a reduction in the fat and protein contents and lipase activity in all the three regions of the diaphragm. In the case of the oxidative enzyme succinic dehydrogenase, however, there was no reduction in its activity in the ventral region but a slight decrease was obtained in the dorsal and lateral regions.

RESULTS

TABLE I: **Showing the effect of starvation on the fat and protein contents and the lipase and succinic dehydrogenase activities in the three regions of the rat diaphragm. ***

Region of the diaphragm	Control				Experimental sample			
	Fat gm % dry wt.	Protein gm % wet wt.	Lipase μl CO_2/mg. Protein/hr.	SDH μg. Formazan/mg. dry wt./2 hrs.	Fat gm % dry wt.	Protein gm % wet wt.	Lipase μl CO_2/mg. Protein/hr.	SDH μg Formazan/mg dry wt./2 hrs.
Dorsal	16.19±1.43	19.83±0.52	41.54±5.52	10.97±2.45	9.99±0.43	15.50±1.82	27.98±5.245	10.05±2.16
Ventral	21.4±0.80	21.42±2.11	29.79±3.11	8.06±2.81	15.82±3.65	18.11±2.8	19.08±2.31	8.13±1.10
Lateral	13.17±1.55	22.33±2.53	24.55±4.25	14.16±3.22	11.53±2.52	15.01±1.26	21.95±2.81	12.25±3.96

* The value obtained in each case is the average of four experiments.

Region of the diaphragm	Actual reduction				Percentage reduction			
	Fat	Protein	Lipase	SDH	Fat	Protein	Lipase	SDH
Dorsal	6.2	4.33	13.56	0.92	38.29	21.83	32.64	8.39
Ventral	5.58	3.31	10.71	—	26.11	15.45	35.95	—
Lateral	1.64	7.32	2.60	1.91	12.45	32.78	10.55	13.49

The ventral and dorsal regions of the diaphragm were found to have a high fat content and during starvation the reduction in fat was also high in these regions. The lowest fat content and the lowest reduction in fat during starvation was seen in the lateral region. The same was true for lipase activity and its reduction during starvation in these three regions. On the other hand the SDH activity was found to be the highest and undergoing relatively more reduction in the lateral region. The lowest SDH activity was in the ventral region and no reduction was observed there. As for protein, the lateral region had the highest protein content and this region also showed the highest reduction in protein during starvation. In the dorsal and ventral regions with considerably lower protein contents, reduction in protein was also less.

The present observations show that during starvation fat and protein are both sources of energy for the diaphragm and that their reduction in amount depends on the quantity of the stored material available in any particular region. In the ventral and dorsal regions therefore, it is fat that is mainly tapped, whereas in the lateral region it is protein.

Goldshtein and Katkova (1935) showed that there is no decrease in the lipase activity of the liver, kindney and lung during starvation. Villa *et al.* (1955) showed that there was no reduction in SDH activity in the liver, heart, brain and kidney and O'Dell *et al.* (1955) observed that the cytochrome oxidase activity in the liver during starvation was not reduced. Our observations on the diaphragm show that lipase activity decreased considerably in the dorsal and ventral regions where there is high fat content and lipase activity. While in the lateral region where there is little fat and low lipase the decrease in lipase activity was very meagre. This suggests that the protein of the enzyme lipase is linked with the lipid. Recently Hollenberg (1960) has shown that lipoprotein lipase activity is considerably reduced in the rat heart and diaphragm during starvation but by treatment with heparin, is again progressively increased.

In the case of SDH there was no reduction in the ventral region and in the other two regions the reduction was not considerable. In the dorsal region there was a high reduction in lipase activity (32.64%) as well as reduction in protein content (21.83%), while in the ventral region there was the highest reduction in lipase (35.95%) and lowest in protein (15.45%). In the lateral region the reduction in lipase was extremely low (10.55%) but that in protein was very high (32.78%). This indicates that in the lateral region there is more non-enzymic protein than in the

other two regions and that this protein is considerably reduced in starvation. From the above observations it also appears that the ventral region is one where fat is stored but little utilized while the dorsal is one where fat is not only present in appreciable quantity but could also be utilized there. The lateral region on the other hand could mobilize some fat and a large amount of protein and also utilize both these metabolites.

Summary

The effect of starvation on the fat and protein contents and the lipase and the succinic dehydrogenase activities in the three regions of the rat diaphragm was studied. The dorsal and ventral regions having high concentrations of fat and lipase showed the highest reduction in fat and lipase activity during starvation. The lateral region having the lowest fat content and lipase activity showed the least amount of reduction in them. With regard to protein and succinic dehydrogenase, the highest concentration as well as reduction in these during starvation was found to be in the lateral region. The significance of the findings has been discussed.

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LOSS OF LIPASE ACTIVITY ON STORAGE OF CELL PARTICULATE FRACTIONS OF THE PIGEON HEART MUSCLE

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DURING a study of the lipase activity in the particulate fractions of the pigeon heart muscle, it was noticed that the mitochondrial suspension stored at 4°C lost its enzyme activity considerably. It was also found that the lipase activity of the uncentrifuged heart muscle homogenate was not affected by storage to any considerable extent. These observations prompted us to study the effect of storage on the lipase activity of the different particulate fractions.

Isolation of the heart muscle cell components: The method for the isolation of the different particulate fractions was adapted from earlier methods for the heart muscle (Plaut and Plaut, 1952; Harman and Feigelson, 1952; Cleland and Slater, 1953; Montgomery and Webb, 1956) with certain modifications. All the preparative operations were done at 0 to 4°C. Hearts were excised from freshly decapitated pigeons and the blood was blotted with filter paper. The auricles were cut off and the adipose tissue on the ventricles was removed because of its high lipase activity (George and Eapen, 1958). The tissue pooled from two adult laboratory pigeons was immediately weighed, washed well with ice-cold 0.25 M sucrose solution and a 10% homogenate in 0.25 M sucrose was prepared by homogenizing for 5 minutes in a previously chilled mortar kept on cracked ice. A part of the homogenate was stored and the rest was centrifuged in an "MSE Superspeed 25" refrigerated centrifuge at 200 × *g*. for 3 minutes. The viscous supernatant phase was poured off and saved for further centrifugation, the residue again resuspended in 0.25 M sucrose and rehomogenized for 5 minutes. It was again centrifuged for 3 minutes at 200 × *g*. and the supernatant obtained was mixed with the first supernatant. The residue was resuspended in 0.25 M sucrose and stored. The combined supernatant was centrifuged for 10 minutes at 600 × *g*. The residue was mixed with the first residue, labelled as Fraction 1 and stored. (This frac-

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tion consisted mainly of myofibrils and cell debris, but some of the other particulates were also present). For the isolation of mitochondria, the supernatant obtained earlier was centrifuged at 9000 × *g*. for 15 minutes, the supernatant decanted off and saved for further centrifugation, the residue washed by homogenization and centrifugation for 10 minutes at 9000 × *g*. and the sediment resuspended in sucrose. This fraction, labelled as Fraction 2 and stored could stain vitally with dilute Janus Green B. The mitochondria in it were spherical in shape. The mixed supernatant was centrifuged at 50,000 × *g*. for 45 minutes and the reddish-brown sediment was washed, resuspended in 0.25 M sucrose, labelled as Fraction 3 and stored. Microscopical examination showed that mitochondria were absent in this fraction. The clear supernatant was labelled as Fraction 4.

All the different particulate fractions and the uncentrifuged whole homogenate were diluted in cold 0.25 M sucrose in such a way that all the dilutions were equal and their lipolytic activities were immediately determined manometrically in a bicarbonate-carbon dioxide buffer system of pH 7.4 at 37°C using the Warburg Apparatus (adapted from Martin and Peers, 1953). 4% (v/v) tributyrin in 0.0148 M $NaHCO_3$ emulsified by shaking with a drop of "Tween 80" was used as substrate.

Aliquots of the different fractions were stored at 4°C and also at −15°C. The lipase activity of the original homogenate and the different fractions were determined after different time intervals. After 12 hours at 4°C the lipase activity of the original homogenate and supernatant (Fraction 4) was not changed, but Fraction 1 was inhibited by 10%. A more detailed study was made on Fractions 2 and 3. Original enzyme activity of Fraction 3 was about twice that of the mitochondrial fraction (Fraction 2). After 5 hours at 4°C the mitochondrial fraction was inhibited by 27% and the microsomal fraction (Fraction 3) by 10%. When the time of storage was increased the loss of activity was not proportional; the loss of activity of the mitochondrial fraction became low. These two fractions which were frozen and stored at −15°C for 5 hours, were inhibited equally in both cases (5%) and the inhibition at any time interval was less than that of those which were kept in liquid state.

It is known that the quantitative determination of enzyme activity in a tissue is dependent not only upon the method for measuring the activity, but also upon the method of preparation of the tissue for analysis (Bonting and Rosenthal, 1960). The present results show that quantita-

tive determination of lipase activity in cell particulate fractions when stored is also dependent on the temperature and time lag. This explains the need for immediate enzyme assay in cell particulates after centrifugation. 95-98% lipase activity in the fractions was recovered when the enzyme activity was determined immediately. The percentage of recovery based on the activity of the original homogenate would go down if kept for long, since the loss of activity of the homogenate is only negligible and that of the fractions quite considerable.

That the nature of succinoxidase in the mitochondria and microsomes is different is shown by Duve *et al* (1955). It is probable that the properties of the mitochondrial and microsomal lipase also may differ since it is now known that the microsomes are the main active sites for fat synthesis and mitochondria for fat utilization. Studies in that direction are in progress in our laboratories.

Summary

The pigeon heart muscle cell components were isolated by differential centrifugation and the effects of temperature and storage on the lipase activity of these fractions were studied.

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LIPASE ACTIVITY OF THE RAT HEART MUSCLE DURING POST-NATAL DEVELOPMENT

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HEART utilizes fat as one of the major sources of energy for its contraction (Visscher, 1938; Bing *et al*, 1954). The occurrence of high concentrations of the enzyme lipase (George and Scaria, 1956) which could hydrolyze fat into fatty acids and glycerol as the first step in the actual utilization of fat involving the oxidation of fatty acids thus liberated has been shown. George and Iype (1959) showed that the heart rate and the level of lipase activity in the heart muscle of the developing chick, are directly related. Here are reported some observations made with a view to see whether such a correlation exists in the rat during post-natal development.

Material and Methods

Albino rats of the Haffkine Institute strain, bred in the laboratory on a standard diet were utilized for the study. Only the males were sacrificed. In the case of new borns, the males were identified by measuring the distance between the anus and the genital papilla (Farris, 1954). In all the cases the hearts were excised after decapitating the animal and were blotted free of blood with the aid of a filter paper. The adipose tissue on the heart was carefully removed and only the ventricles were used. Hearts from 5 to 6 individuals were pooled for each experiment. The tissue after having been weighed quickly was homogenized in cold distilled water and a 2 5% homogenate was prepared. The lipase activity of the homogenate was determined manometrically in a bicarbonate carbon dioxide buffer system of pH 7.4 (adapted from Martin and Peers, 1953). The reaction flask contained 1.5 ml. of 0.025 M sodium bicarbonate and 1 ml. of the homogenate in the main chamber and 0.5 ml. of the substrate (4% v/v tributyrin in 0.0148 M sodium bicarbonate emulsified with a drop of "Tween 80") in the side arm. The flask and manometer were gassed with a mixture of 5% CO_2 and 95% N_2 for 3 minutes and equilibrated for

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10 minutes at 37°C. The substrate was then tipped in and after another 3 minutes of equilibration readings were taken at regular intervals for one hour. The lipase activity is expressed as the number of μl. CO_2/mg. protein/hour. Protein was estimated according to the micro-Kjeldahl steam distillation method (Hawk *et al*, 1954).

Results and Discussion

The lipase activity in the heart of rats in post-natal development was found to increase in the first two weeks and showed a gradual decrease as they grew older (Table 1). The heart lipase activity of the adult rat is

TABLE 1

Lipase Activity of the Rat Heart Muscle during Post-natal Development

Age	Lipase activity (μl. CO_2/mg. protein/hour)	Number of experiments
New born	23.68	6
1 week	31.67	5
2 weeks	33.94	5
3 weeks	33.53	4
4 weeks	32.86	4
6 weeks	31.98	4
Adult	29.38	4

lower than that of the two weeks old young ones. Since the lipase activity is computed per mg. protein, any fluctuations, if any, in the percentage of protein in the heart muscle could make the lipase value deceptive. The protein content of the heart muscle during post-natal development also was estimated and no fluctuations in the protein content were detected. Electrocardiographic observations on the rat young ones were attempted, but it was not possible to register their heart beat. Even though the heart rate in the post-natal development of the rat is not known, however, from the general activity of the animal it could be said that the two weeks old young ones have a higher heart rate. This increased activity of the heart is to be expected since this is the time at which the eyes open and they move about actively. The heart lipase activity also was found to be higher at this time. It is known that the heart rate increases with the increase in the basal metabolic rate. The early infant rats on the other hand are very

passive and the lipase activity in the heart is also comparatively low. The decline in the lipase activity in the heart of the adults could be attributed to aging which is known to decrease the heart rate.

Summary

Lipase activity in the heart muscle of adult rats as well as young ones during post-natal development was determined manometrically. It was found that the lipase values obtained could be correlated with the heart rate.

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